

At the high dose (1.2  $\mu$ g), the glycoPEGylated rFSH had somewhat higher *in vivo* activity than the unPEGylated rFSH.

### G-CSF

#### 28. GlycoPEGylation of G-CSF produced in CHO cells

5 **Preparation of Asialo-Granulocyte-Colony Stimulation Factor (G-CSF).** G-CSF produced in CHO cells is dissolved at 2.5 mg/mL in 50 mM Tris 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, 5 mM CaCl<sub>2</sub> and concentrated to 500  $\mu$ L in a Centricon Plus 20 centrifugal filter. The solution is incubated with 300 mU/mL Neuraminidase II (*Vibrio cholerae*) for 16  
10 hours at 32 °C. To monitor the reaction a small aliquot of the reaction is diluted with the appropriate buffer and a IEF gel performed. The reaction mixture is then added to prewashed N-(*p*-aminophenyl)oxamic acid-agarose conjugate (800  $\mu$ L/mL reaction volume) and the washed beads gently rotated for 24 hours at 4 °C. The mixture is centrifuged at 10,000 rpm and the supernatant was collected. The beads are washed 3 times with Tris-EDTA buffer,  
15 once with 0.4 mL Tris-EDTA buffer and once with 0.2 mL of the Tris-EDTA buffer and all supernatants are pooled. The supernatant is dialyzed at 4 °C against 50 mM Tris -HCl pH 7.4, 1 M NaCl, 0.05% NaN<sub>3</sub> and then twice more against 50 mM Tris -HCl pH 7.4, 1 M NaCl, 0.05% NaN<sub>3</sub>. The dialyzed solution is then concentrated using a Centricon Plus 20  
20 centrifugal filter and stored at -20 °C. The conditions for the IEF gel were run according to the procedures and reagents provided by Invitrogen. Samples of native and desialylated G-CSF are dialyzed against water and analyzed by MALDI-TOF MS.

25 **Preparation of G-CSF-(alpha2,3)-Sialyl-PEG.** Desialylated G-CSF was dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of ST3Gal1 at 32°C for 2 days.  
To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction had CMP-  
SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas  
30 G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis

according to the procedures and reagents supplied by Invitrogen. Samples of native and PEGylated G-CSF are dialyzed against water and analyzed by MALDI-TOF MS.

**Preparation of G-CSF-(alpha2,3)-Sialyl-PEG.** G-CSF produced in CHO cells, which contains an alpha2,3-sialylated O-linked glycan, is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of CST-II at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction has CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples of native and PEGylated G-CSF are dialyzed against water and analyzed by MALDI-TOF MS.

**Preparation of G-CSF-(alpha2,6)-Sialyl-PEG.** G-CSF, containing only O-linked GalNAc, is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of ST6GalNAcI or II at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction has CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples of native and PEGylated G-CSF are dialyzed against water and analyzed by MALDI-TOF MS.

G-CSF produced in CHO cells was treated with Arthrobacter sialidase and was then purified by size exclusion on Superdex75 and was treated with ST3Gal1 or ST3 Gal2 and then with CMP-SA-PEG 20Kda. The resulting molecule was purified by ion exchange and

gel filtration and analysis by SDS PAGE demonstrated that the PEGylation was complete. This is the first demonstration of glycoPEGylation of an O-linked glycan.

Glucocerebrosidase

5                   29. Glucocerebrosidase-mannose-6-phosphate produced in CHO cells

This example sets forth the procedure to glycoconjugate mannose-6-phosphate to a peptide produced in CHO cells such as glucocerebrosidase.

10                  **Preparation of asialo-glucoceramidase.** Glucocerebrosidase produced in CHO cells is dissolved at 2.5 mg/mL in 50 mM Tris 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, and is incubated with 300 mU/mL sialidase-agarose conjugate for 16 hours at 32 °C. To monitor the reaction a small aliquot of the reaction is diluted with the appropriate buffer and a IEF gel and SDS-PAGE performed according to Invitrogen procedures. The mixture is centrifuged at 10,000 rpm and the supernatant is collected. The beads are washed 3 times with Tris-EDTA buffer, once with 0.4 mL Tris-EDTA buffer, and once with 0.2 mL of the Tris-EDTA buffer.

15                  All supernatants are pooled. The supernatant is dialyzed at 4 °C against 50 mM Tris-HCl pH 7.4, 1 M NaCl, 0.05% NaN<sub>3</sub> and then twice more against 50 mM Tris-HCl pH 7.4, 1 M NaCl, 0.05% NaN<sub>3</sub>. The dialyzed solution is then concentrated using a Centricon Plus 20 centrifugal filter. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed 20 against water and analyzed by MALDI-TOF MS.

**Preparation of Glucocerebrosidase-SA-linker-Mannose-6-phosphate (procedure**

1). Asialo-glucocerebrosidase from above is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-linker-Man-6-phosphate and 0.1 U/mL of ST3Gal3 at 32°C for 2 days. To monitor the incorporation of sialic acid-linker-Man-6-phosphate, a small aliquot of the reaction had CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas TSK-Gel-3000 analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. When the reaction is complete, the reaction mixture is purified 25 using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using

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SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

**Preparation of Glucocerebrosidase-SA-linker-Mannose-6-phosphate (procedure 2).** Glucocerebrosidase, produced in CHO but incompletely sialylated, is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-linker-Man-6-phosphate and 0.1 U/mL of ST3Gal3 at 32°C for 2 days. To monitor the incorporation of sialic acid-linker-Man-6-phosphate, a small aliquot of the reaction had CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas TSK-Gel-3000 analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. When the reaction is complete, the reaction mixture is purified using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

### 30. Glucocerebrosidase-transferrin

This example sets forth the procedures for the glycoconjugation of proteins, and in particular, transferrin is glycoconjugated to glucocerebrosidase. The GlcNAc-ASN structures are created on glucoceraminidase, and Transferrin-SA-Linker-Gal-UDP is conjugated to GNDF GlcNAc-ASN structures using galactosyltransferase.

**Preparation of GlcNAc-glucocerebrosidase (Cerezyme™).** Cerezyme™ (glucocerebrosidase) produced in CHO cells is dissolved at 2.5 mg/mL in 50 mM Tris 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, and is incubated with 300 mU/mL Endo-H-agarose conjugate for 16 hours at 32 °C. To monitor the reaction a small aliquot of the reaction is diluted with the appropriate buffer and a IEF gel and SDS-PAGE performed according to Invitrogen procedures. The mixture is centrifuged at 10,000 rpm and the supernatant is collected. The beads are washed 3 times with Tris-EDTA buffer, once with 0.4 mL Tris-EDTA buffer and once with 0.2 mL of the Tris-EDTA buffer and all supernatants are pooled. The supernatant is dialyzed at 4 °C against 50 mM Tris -HCl pH 7.4, 1 M NaCl, 0.05% NaN<sub>3</sub> and then twice

more against 50 mM Tris -HCl pH 7.4, 1 M NaCl, 0.05% NaN<sub>3</sub>. The dialyzed solution is then concentrated using a Centricon Plus 20 centrifugal filter. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF

5 MS.

Preparation of Transferrin-SA-Linker-Gal-glucocerebrosidase. Transferrin-SA-Linker-Gal-UDP from above is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 5 mM MnCl<sub>2</sub>, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 2.5 mg/mL GlcNAc-glucocerebrosidase and 0.1 U/mL of galactosyltransferase at 32°C for 2 days. To monitor the incorporation of glucocerebrosidase, the peptide is separated by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1) and the product detected by UV absorption. The reaction mixture is then purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

#### GM-CSF

##### 31. Generation and PEGylation of GlcNAc-ASN Structures: GM-CSF produced in *Saccharomyces*

20 This example sets forth the preparation of Tissue-type Activator with PEGylated GlcNAc-Asn structures.

Recombinant GM-CSF expressed in yeast is expected to contain 2 N-linked and 2 O-linked glycans. The N-linked glycans should be of the branched mannan type. This recombinant glycoprotein is treated with an endoglycosidase from the group consisting of endoglycosidase H, endoglycosidase-F1, endoglycosidase-F2, endoglycosidase-F3, endoglycosidase-M either alone or in combination with mannosidases I, II and III to generate GlcNAc nubs on the asparagine (Asn) residues on the peptide/protein backbone.

30 The GlcNAc-Asn structures on the peptide/protein backbone is then be modified with galactose or galactose-PEG using UDP-galactose or UDP-galactose-6-PEG, respectively, and a galactosyltransferase such as GalT1. In one case the galactose-PEG is the terminal residue.

In the second case the galactose is further modified with SA-PEG using a CMP-SA-PEG donor and a sialyltransferase such as ST3GalIII. In another embodiment the GlcNAc-Asn structures on the peptide/protein backbone can be galactosylated and sialylated as described above, and then further sialylated using CMP-SA-PEG and an  $\alpha$ 2,8-sialyltransferase such as 5 the enzyme encoded by the *Campylobacter jejuni* cst-II gene.

Herceptin<sup>TM</sup>

32. Glycoconjugation of mithramycin to Herceptin<sup>TM</sup>

This example sets forth the procedures to glycoconjugate a small molecule, such as 10 mithramycin to Fc region glycans of an antibody molecule produced in mammalian cells. Here, the antibody Herceptin<sup>TM</sup> is used, but one of skill in the art will appreciate that the method can be used with many other antibodies.

**Preparation of Herceptin<sup>TM</sup>-Gal-linker-mithramycin.** Herceptin<sup>TM</sup> is dissolved at 15 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 5 mM MnCl<sub>2</sub>, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 1 mM UDP-galactose-linker-mithramycin and 0.1 U/mL of 20 galactosyltransferase at 32°C for 2 days to introduce the mithramycin in the Fc region glycans. To monitor the incorporation of galactose, a small aliquot of the reaction has <sup>14</sup>C-galactose-UDP ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The radioactive label incorporation into the peptide is quantitated using an in-line radiation detector.

When the reaction is complete, the reaction mixture is purified using a Toso Haas 25 TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The fractions containing product are combined, concentrated, buffer exchanged and then freeze-dried. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

Interferon  $\alpha$  and Interferon  $\beta$ 33. GlycoPEGylation of Proteins expressed in Mammalian or Insect Systems:  
EPO, Interferon  $\alpha$  and Interferon  $\beta$ 

5 This example sets forth the preparation of PEGylated peptides that are expressed in mammalian and insect systems.

**Preparation of acceptor from mammalian expression systems.** The peptides to be glycoPEGylated using CMP-sialic acid PEG need to have glycans terminating in galactose. Most peptides from mammalian expression systems will have terminal sialic acid that first needs to be removed.

10 **Sialidase digestion.** The peptide is desialylated using a sialidase. A typical procedure involves incubating a 1 mg/mL solution of the peptide in Tris-buffered saline, pH 7.2, with 5 mM CaCl<sub>2</sub> added, with 0.2 U/mL immobilized sialidase from *Vibrio cholera* (Calbiochem) at 32°C for 24 hours. Microbial growth can be halted either by sterile filtration or the inclusion of 0.02% sodium azide. The resin is then removed by centrifugation or 15 filtration, and then washed to recover entrapped peptide. At this point, EDTA may be added to the solution to inhibit any sialidase that has leached from the resin.

15 **Preparation from insect expression systems.** EPO, interferon-alpha, and interferon-beta may also be expressed in non-mammalian systems such as yeast, plants, or insect cells. The peptides to be glycoPEGylated using CMP-sialic acid PEG need to have 20 glycans terminating in galactose. The majority of the N-glycans on peptides expressed in insect cells, for example, are the trimannosyl core. These glycans are first built out to glycans terminating in galactose before they are acceptors for sialyltransferase.

20 **Building acceptor glycans from trimannosyl core.** Peptide (1 mg/mL) in Tris-buffered saline, pH 7.2, containing 5 mM MnCl<sub>2</sub>, 5 mM UDP-glcNAc, 0.05 U/mL GLCNACT I, 0.05 U/mL GLCNACT II, is incubated at 32°C for 24 hours or until the 25 reaction is substantially complete. Microbial growth can be halted either by sterile filtration or the inclusion of 0.02% sodium azide. After buffer exchange to remove UDP and other small molecules, UDP-galactose and MnCl<sub>2</sub> are each added to 5 mM, galactosyltransferase is added to 0.05 U/mL, and is incubated at 32°C for 24H or until the reaction is substantially 30 complete. Microbial growth can be halted either by sterile filtration or the inclusion of 0.02% sodium azide. The peptides are then ready for glycoPEGylation.

Building O-linked glycans. A similar strategy may be employed for interferon alpha to produce enzymatically the desired O-glycan Gal-GalNAc. If necessary, GalNAc linked to serine or threonine can be added to the peptide using appropriate peptide GalNAc transferases (e.g. GalNAc T1, GalNAc T2, T3, T4, etc. ) and UDP-GalNAc. Also, if needed, 5 galactose can be added using galactosyltransferase and UDP-galactose.

GlycoPEGylation using sialyltransferase. The glycopeptides (1 mg/mL) bearing terminal galactose in Tris buffered saline + 0.02% sodium azide are incubated with CMP-SA-PEG (0.75 mM) and 0.4 U/mL sialyltransferase (ST3Gal3 or ST3Gal4 for N-glycans on EPO and interferon beta; ST3Gal4, or ST3Gal1 for O-glycans on interferon alpha) at 32°C for 24 10 hours. Other transferases that may work include the 2,6 sialyltransferase from *Photobacterium damsella*. The acceptor peptide concentration is most preferably in the range of 0.1 mg/mL up to the solubility limit of the peptide. The concentration of CMP-SA-PEG should be sufficient for there to be excess over the available sites, but not so high as to cause 15 peptide solubility problems due to the PEG, and may range from 50 µM up to 5 mM, and the temperature may range from 2°C up to 40°C. The time required for complete reaction will depend on the temperature, the relative amounts of enzyme to acceptor substrate, the donor substrate concentration, and the pH.

#### 34. GlycoPEGylation of Interferon $\alpha$ produced in CHO cells

Preparation of Asialo-Interferon  $\alpha$ . Interferon alpha produced from CHO cells is 20 dissolved at 2.5 mg/mL in 50 mM Tris 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, 5 mM CaCl<sub>2</sub> and concentrated to 500 µL in a Centricon Plus 20 centrifugal filter. The solution is incubated with 300 mU/mL Neuraminidase II (*Vibrio cholerae*) for 16 hours at 32 °C. To 25 monitor the reaction a small aliquot of the reaction is diluted with the appropriate buffer and a IEF gel performed. The reaction mixture is then added to prewashed N-(*p*-aminophenyl)oxamic acid-agarose conjugate (800 µL/mL reaction volume) and the washed beads gently rotated for 24 hours at 4 °C. The mixture is centrifuged at 10,000 rpm and the supernatant was collected. The beads are washed 3 times with Tris-EDTA buffer, once with 0.4 mL Tris-EDTA buffer and once with 0.2 mL of the Tris-EDTA buffer and all 30 supernatants were pooled. The supernatant is dialyzed at 4 °C against 50 mM Tris -HCl pH 7.4, 1 M NaCl, 0.05% NaN<sub>3</sub> and then twice more against 50 mM Tris -HCl pH 7.4, 1 M

NaCl, 0.05% NaN<sub>3</sub>. The dialyzed solution is then concentrated using a Centricon Plus 20 centrifugal filter and stored at -20 °C. The conditions for the IEF gel are run according to the procedures and reagents provided by Invitrogen. Samples of native and desialylated G-CSF are dialyzed against water and analyzed by MALDI-TOF MS.

5 **Preparation of Interferon-alpha-(alpha2,3)-Sialyl-PEG.** Desialylated interferon-alpha is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of ST3Gal1 at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction had CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide 10 is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions 15 based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples of native and desialylated Interferon-alpha are dialyzed against water and analyzed by MALDI-TOF MS.

15 **Preparation of Interferon-alpha-(alpha2,8)-Sialyl-PEG.** Interferon-alpha produced in CHO, which contains an alpha2,3-sialylated O-linked glycan, is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated 20 with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of CST-II at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction has CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free 25 label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on 30 UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples of native and PEGylated interferon-alpha are dialyzed against water and analyzed by MALDI-TOF MS.

**Preparation of Interferon-alpha-(alpha2,6)-Sialyl-PEG.** Interferon-alpha, containing only O-linked GalNAc, was dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of ST6GalNAcI or II at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction had CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples of native and PEGylated interferon-alpha are dialyzed against water and analyzed by MALDI-TOF MS.

15 35. GlycoPEGylation of Interferon-β-1a with PEG (10 kDa) and PEG (20 kDa)

This example illustrates a procedure PEGylate Interferon-β with either PEG (10 kDa) or PEG (20 kDa).

20 Briefly, Interferon-β-1a (INF-β) was obtained from Biogen (Avonex<sup>TM</sup>). The IFN-β was first purified by Superdex-75 chromatography. The IFN-β was then desialylated with *Vibrio cholerae* sialidase. The INF-β was then PEGylated with SA-PEG (10 kDa) or SA-PEG (20 kDa) and purified with Superdex-200 chromatography.

25 **Superdex-75 chromatography purification.** INF-β (150 µg) was applied to a Superdex-75 column (Amersham Biosciences, Arlington Heights, IL) and eluted with PBS with 0.5 M NaCl, 0.02 Tween-20, 20 mM histidine and 10% glycerol. The eluant was monitored for absorbance at 280 nm (Figure 172A and 172B) and fractions were collected. Peaks 4 and 5 were pooled, concentrated in an Amicon Ultra 15 spin filter (Millipore, Billerica, MA), and the buffer was exchanged to TBS with 5 mM CaCl<sub>2</sub>, 0.02% Tween-20, 20 mM histidine and 10% glycerol.

30 **Sialidase Reaction.** The INF-β was then desialydated with *Vibrio cholera* salidase (70 mU/ml, CALBIOCHEM®, EMD Biosciences, Inc., San Diego, CA) on agarose in TBS

with 5 mM CaCl<sub>2</sub>, 0.02% Tween-20, 20 mM histidine and 10% glycerol. The reaction was carried out at 32°C for 18 hours. The INF-β was removed from the agarose with a 0.22 µm Spin-X™ filter (Corning Technology, Inc., Norcross, GA). Figure 173A depicts the MALDI analysis of glycans released from native INF-β. The native INF-β has many glycoforms containing terminal sialic acid moieties. Figure 173B depicts the MALDI analysis of glycans released from desialylated INF-β. The desialylated INF-β has primarily one glycoform which is bi-antennary with terminal galactose moieties.

**Lectin Dot-Blot Analysis of Sialylation.** Samples of the INF-β from the desialidase reaction were dot-blotted onto nitrocellulose and then blocked with Tris buffered saline (TBS: 0.05M Tris, 0.15M NaCl, pH 7.5) and DIG kit (glycan differentiation kit available from Roche #1 210 238) blocking buffer. Some of the blots were incubated with *Maackia amurensis* agglutinin (MAA) labeled with digoxigenin (DIG) (Roche Applied Science, Indianapolis, IL) to detect α2,3-sialylation of INF-β. These blots were washed with TBS then incubated with anti-digitonin antibody labeled with alkaline phosphatase, then washed again with TBS and developed with NBT/X-phosphate solution, wherein NBT is 4-nitro blue tetrazolium chloride and X-phosphate is 5-bromo-4-chloro-3-indoyl phosphate. The left side of Figure 174 depicts the results of the MAA blot of INF-β after the desialylation reaction. The INF-β is partially desialylated, as indicated by the decrease in dot development as compared to native INF-β in the desialylated samples.

Other blots were incubated with *Erthrina cristagalli* lectin (ECL) labeled with biotin (Vector Laboratories, Burlingame, CA) to detect exposed galactose residues on INF-β. After incubation with 2.5 µg/ml ECL, the blots were washed in TBS and incubated with streptavidin labeled with alkaline phosphatase. The blots were then washed again and developed. The right side of Figure 174 depicts the ECL blot after development. The increased intensity of the dot of desialylated INF-β as compared to the native INF-β indicate more exposed galactose moieties and therefore extensive desialylation.

**PEGylation of Desialylated INF-β with SA-PEG (10 kDa).** Desialylated INF-β (0.05 mg/ml) was PEGylated with ST3Gal3 (50 mU/ml) and CMP-SA-PEG (10 kDa) (250 µM) in an appropriate buffer of TBS + 5 mM CaCl<sub>2</sub>, 0.02% Tween 20, 20 mM histidine, 10%

glycerol for 50 hours at 32°C. Figure 175 depicts the SDS-PAGE analysis of the reaction products showing PEGylated INF- $\beta$  at approximately 98 kDa.

5 **PEGylation of Desialylated INF- $\beta$  with SA-PEG (20 kDa).** Desialylated INF- $\beta$  (0.5 mg/ml) was PEGylated with ST3Gal3 (170 mU/ml) and CMP-SA-PEG (20 kDa) in an appropriate buffer of TBS + 5 mM CaCl<sub>2</sub>, 0.02% Tween 20, 20 mM histidine, 10% glycerol for 50 hours at 32°C. Figure 176 depicts the SDS-PAGE analysis the products of the PEGylation reaction. The PEGylated INF- $\beta$  has many higher molecular weight bands not found in the unmodified INF- $\beta$  indicating extensive PEGylation.

10 **Superdex-200 Purification of INF- $\beta$  PEGylated with PEG (10 kDa).** The products of the PEGylation reaction were separated on a Superdex-200 column (Amersham Biosciences, Arlington Heights, IL) in PBS with 0.5 NaCl, 0.02 Tween-20, 20 mM histidine and 10% glycerol at 1ml/min and 30 cm/hr flow. The eluant was monitored for absorbance at 280 nm (Figure 177) and fractions were collected. Peaks 3 and 4 were pooled and concentrated in an Amicon Ultra 15 spin filter.

15 **Bioassay of INF- $\beta$  PEGylated with PEG (10 kDa).**

The test is inhibition of the proliferation of the lung carcinoma cell line, A549. The A549 cell line are lung carcinoma adherent cells growing in RPMI + 10% FBS at 37°C 5% CO<sub>2</sub>. They can be obtained from ATCC # CCL-185. Wash the cells with 10 ml of PBS and remove the PBS. Add 5 ml of trypsin, incubate for 5 minutes at room temperature or 2 minutes at 37°C. When the cells are detached resuspend into 25 ml of media and count the cells. Dilute the cells at a concentration of 10000 cells/ml and add 200  $\mu$ l / well (96 wells plate). Incubate for 4 hours at 37°C 5% CO<sub>2</sub>. Prepare 1 ml of IFN B at a concentration of 0.1  $\mu$ g/ml. Filter it under the hood with a 0.2  $\mu$ m filter. Add 100  $\mu$ l per well (8 replicates = 1 lane). Incubate for 3 days (do not let the cells go to confluence). Remove 200  $\mu$ l of media (only 100ul per well left). Add 25  $\mu$ l of MTT (Sigma) (5 mg/ml filtered 0.22 $\mu$ m). Incubate for 4 hours at 37°C and 5% CO<sub>2</sub>. Aspirate the media gently and add 100  $\mu$ l of a mixture of isopropanol (100 ml and 6N HCl. Aspirate up and down to homogenize the crystal violet. Read OD 570nm (remove the background at 630 or 690 nm).

30 Figure 178 depicts the results of the bioassay of the peaks containing INF- $\beta$  PEGylated with PEG (10 kDa) as eluted from the Superdex-200 column.

5 **Superdex-200 Purification of INF- $\beta$  PEGylated with PEG (20 kDa).** The products of the PEG (20 kDa) PEGylation reaction were separated on a Superdex-200 column (Amersham Biosciences, Arlington Heights, IL) in PBS with 0.5 NaCl, 0.02 Tween-20, 20 mM histidine and 10% glycerol at 1 ml/min flow. The eluant was monitored for absorbance at 280 nm (Figure 179) and fractions were collected. Peak 3 contained most of the INF- $\beta$  PEGylated with PEG (20 kDa).

10 **Endotoxin test of INF- $\beta$  PEGylated with PEG (20 kDa).**

Limulus Lysate Test was performed, BioWhittaker # 50-647U

Table 24. Results of the endotoxin test of INF- $\beta$  PEGylated with PEG (20 kDa).

Concentration			
INF- $\beta$ with PEG (20 kDa)	10 EU/ml	0.06 mg/ml	0.16 EU/ $\mu$ g
INF- $\beta$ with PEG (20 kDa)	1 EU/ml	0.07 mg/ml	0.014 EU/ $\mu$ g
Native INF- $\beta$	40 EU/ml	0.1 mg/ml	0.4 EU/ $\mu$ g

15 Remicade<sup>TM</sup>

36. GlycoPEGylation of Remicade<sup>TM</sup> antibody

This example sets forth the procedure to glycoPEGylate a recombinant antibody molecule by introducing PEG molecules to the Fc region glycans. Here Remicade<sup>TM</sup>, a TNF-15 R:IgG Fc region fusion protein, is the exemplary peptide.

20 **Preparation of Remicade<sup>TM</sup>-Gal-PEG (10 kDa).** Remicade<sup>TM</sup> is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 5 mM MnCl<sub>2</sub>, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 1 mM UDP-galactose-PEG (10 kDa) and 0.1 U/mL of galactosyltransferase at 32°C for 2 days to introduce the PEG in the Fc region glycans. To monitor the incorporation of galactose, a small aliquot of the reaction has <sup>14</sup>C-galactose-UDP ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The radioactive label incorporation into the peptide is quantitated using an in-line radiation detector.

25 When the reaction is complete, the reaction mixture is purified using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The fractions containing product are combined, concentrated, buffer

exchanged and then freeze-dried. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

5 Rituxan™

37. Glycoconjugation of geldanamycin to Rituxan™

This example sets forth the glycoconjugation of a small molecule, such as geldanamycin, to the Fc region glycans of an antibody produced in CHO cells, such as Rituxan™. Here, the antibody Rituxan™ is used, but one of skill in the art will appreciate 10 that the method can be used with many other antibodies.

Preparation of Rituxan™-Gal-linker-geldanamycin. Rituxan™ is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 5 mM MnCl<sub>2</sub>, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 1 mM UDP-galactose-linker-geldanamycin and 0.1 U/mL of galactosyltransferase at 32°C for 2 days to introduce the geldanamycin in the Fc region 15 glycans. To monitor the incorporation of galactose, a small aliquot of the reaction has <sup>14</sup>C-galactose-UDP ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The radioactive label incorporation into the peptide is quantitated using an in-line radiation detector.

20 When the reaction is complete, the reaction mixture is purified using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The fractions containing product are combined, concentrated, buffer exchanged and then freeze-dried. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples 25 are dialyzed against water and analyzed by MALDI-TOF MS.

Rnase

38. Remodeling high mannose N-glycans to hybrid and complex N-glycans:  
Bovine pancreatic RNase

30 This example sets forth the preparation of bovine pancreas RNase with hybrid or complex N-glycans. The high mannose N-linked glycans of the RNase are enzymatically

digested and elaborated to create hybrid N-linked glycans. Additionally, the high mannose N-linked glycans of the RNase are enzymatically digested and elaborated to create complex N-linked glycans.

High mannose structures of *N*-linked oligosaccharides in glycopeptides can be modified to hybrid or complex forms using the combination of  $\alpha$ -mannosidases and glycosyltransferases. This example summarizes the results in such efforts using a simple *N*-Glycan as a model substrate.

Ribonuclease B (RNaseB) purified from bovine pancreas (Sigma) is a glycopeptide consisting of 124 amino acid residues. It has a single potential *N*-glycosylation site modified with high mannose structures. Due to its simplicity and low molecular weight (13.7 kDa to 15.5 kDa), ribonuclease B is a good candidate to demonstrate the feasibility of the *N*-Glycan remodeling from high mannose structures to hybrid or complex *N*-linked oligosaccharides. The MALDI-TOF spectrum of RNaseB (Figure 180A) and HPLC profile for the oligosaccharides cleaved from RNaseB by N-Glycanase (Figure 180B) indicated that, other than a small portion of the non-modified peptide, the majority of *N*-glycosylation sites of the peptide are modified with high mannose oligosaccharides consisting of 5 to 9 mannose residues.

**Conversion of high mannose *N*-Glycans to hybrid *N*-Glycans.** High mannose *N*-Glycans were converted to hybrid *N*-Glycans using the combination of  $\alpha$ 1,2-mannosidase, GlcNAcT-I ( $\beta$ -1,2-*N*-acetyl glucosaminyl transferase), GalT-I ( $\beta$ 1,4-galactosyltransferase) and  $\alpha$ 2,3-sialyltransferase /or  $\alpha$ 2,6-sialyltransferase as shown in Figure 181.

As an example, high mannose structures in RNaseB were successfully converted to hybrid structures.

$\text{Man}_5\text{GlcNAc}_2\text{-R}$  was obtained from  $\text{Man}_{5-9}\text{GlcNAc}_2\text{-R}$  catalyzed by a single  $\alpha$ 1,2-mannosidase cloned from *Trichoderma reesei* (Figure 182). RNase B (1 g, about 67  $\mu\text{mol}$ ) was incubated at 30°C for 45 hr with 15 mU of the recombinant *T. reesei*  $\alpha$ 1,2-mannosidase in MES buffer (50 mM, pH 6.5) in a total volume of 10 mL.  $\text{Man}_{6-9}\text{GlcNAc}_2$ -protein structures have been successfully converted to  $\text{Man}_5\text{GlcNAc}_2$ -protein with high efficiency by the recombinant mannosidase.

Alternately,  $\text{Man}_5\text{GlcNAc}_2\text{-R}$  was obtained from  $\text{Man}_{5.9}\text{GlcNAc}_2\text{-R}$  catalyzed by a single  $\alpha$ 1,2-mannosidase purified from *Aspergillus saitoi* (Figure 183). RNase B (40  $\mu\text{g}$ , about 2.7 nmol) was incubated at 37°C for 42.5 hr with 25  $\mu\text{U}$  of the commercial *A. saitoi*  $\alpha$ 1,2-mannosidase (Glyko or CalBioChem) in NaOAC buffer (100 mM, pH 5.0) in a total 5 volume of 20  $\mu\text{l}$ .  $\text{Man}_{6.9}\text{GlcNAc}_2$ -protein structures were successfully converted to  $\text{Man}_5\text{GlcNAc}_2$ -protein by the commercially available mannosidase. However, a new peak corresponding to the GlcNAc-protein appears in the spectrum, indicating the possible contamination of endoglycosidase H in the preparation. Although several mammalian alpha-mannosidases were required to achieve this step, the fungal  $\alpha$ 1,2-mannosidase was very 10 efficient to remove all  $\alpha$ 1,2-linked mannose residues.

GlcNAcT-I then added a GlcNAc residue to the  $\text{Man}_5\text{GlcNAc}_2\text{-R}$  (Figure 184). The reaction mixture after the *T. reesei*  $\alpha$ 1,2-mannosidase reaction containing RNase B (600  $\mu\text{g}$ , about 40 nmol) was incubated with non-purified recombinant GlcNAcT-I (34 mU) in MES buffer (50 mM, pH 6.5) containing  $\text{MnCl}_2$  (20 mM) and UDP-GlcNAc (5 mM) in a total 15 volume of 400  $\mu\text{l}$ . at 37°C for 42 hr. A GlcNAc residue was quantitatively added to  $\text{Man}_5\text{GlcNAc}_2$ -protein by the recombinant GlcNAcT-I.

A Gal residue was then added using GalT 1 (Figure 185). The reaction mixture after the GnT-I reaction containing RNase B (120  $\mu\text{g}$ , about 8 nmol) was incubated at 37°C for 20 hr with 3.3 mU of the recombinant GalT-1 in Tris-HCl buffer (100 mM, pH 7.3) containing 20 UDP-Gal (7.5 mM) and  $\text{MnCl}_2$  (20 mM) in a total volume of 100  $\mu\text{l}$ . A Gal residue was added to about 98% of the GlcNAc- $\text{Man}_5\text{GlcNAc}_2$ -protein by the recombinant GalT 1.

The next step was the addition of a sialic acid using an  $\alpha$ 2,3-sialyltransferase or an  $\alpha$ 2,6-sialyltransferase (Figure 186). As an example, ST3Gal III, an  $\alpha$ 2,3-sialyltransferase was used. The reaction mixture after the GalT-1 reaction containing RNase B (13  $\mu\text{g}$ , about 0.87 nmol) was incubated at 37°C for 16 hr with 8.9 mU of recombinant ST3Gal III in Tris-HCl 25 buffer (100 mM, pH 7.3) containing CMP-Sialic acid (5 mM) and  $\text{MnCl}_2$  (20 mM) in a total volume of 20  $\mu\text{l}$ . A sialic acid residue was added to about 90% of the Gal-GlcNAc- $\text{Man}_5\text{GlcNAc}_2$ -protein by recombinant ST3Gal III using CMP-SA as the donor. The yield can be further improved by adjusting the reaction conditions.

For convenience, no purification or dialysis step was required after each reaction described above. More interesting, GalT 1 and ST3Gal III can be combined in a one-pot reaction. Similar yields were obtained as compared with the separate reactions. The reaction mixture after the GlcNAcT-I reaction containing RNase B (60  $\mu$ g, about 4 nmol) was 5 incubated at 37°C for 20 hr with 1.7 mU of recombinant GalT 1, 9.8 mU of recombinant ST3Gal III in Tris-HCl buffer (100 mM, pH 7.3) containing UDP-Gal (7.5 mM), CMP-sialic acid (5 mM) and MnCl<sub>2</sub> (20 mM) in a total volume of 60  $\mu$ l.

As shown in Figure 187, SA-PEG (10 kDa) was successfully added to the RNaseB. The reaction mixture after the GalT-1 reaction containing RNase B (6.7  $\mu$ g, about 0.45 nmol) 10 was dialyzed against H<sub>2</sub>O for 1 hour at room temperature and incubated at 37°C for 15.5 hours with 55 mU of the recombinant ST3Gal III in Tris-HCl buffer (50 mM, pH 7.3) containing CMP-SA-PEG (10 kDa) (0.25 mM) and MnCl<sub>2</sub> (20 mM) in a total volume of 20  $\mu$ l. PEG-modified sialic acid residues were successfully added to the Gal-GlcNAc- 15 Man<sub>5</sub>GlcNAc<sub>2</sub>-peptide by the recombinant ST3Gal III. The yield can be further improved by adjusting the reaction conditions.

**Conversion of high mannose N-Glycans to complex N-Glycans.** To achieve this conversion, a GlcNAc $\beta$ 1,2Man<sub>3</sub>GlcNAc<sub>2</sub>-peptide intermediate is obtained. As shown in Figure 188, there are at least four feasible routes to carry out the reaction from Man<sub>5</sub>GlcNAc<sub>2</sub>-peptide to this intermediate:

20 **Route I:** The Man<sub>5</sub>GlcNAc<sub>2</sub>-peptide produced by the fungal  $\alpha$ 1,2 mannosidase is a substrate of GlcNAc transferase I (GlcNAcT-I, enzyme 2) which adds one GlcNAc. The terminal  $\alpha$ 1,3- and  $\alpha$ 1,6-linked mannose residues of GlcNAcMan<sub>5</sub>GlcNAc<sub>2</sub>-peptide is removed by Golgi  $\alpha$ -mannosidase II (ManII, enzyme 5). This route is a part of the natural pathway for the processing of *N*-linked oligosaccharides carried out in higher organisms.

25 **Route II:** Two mannose residues are first removed by an  $\alpha$ -mannosidase (enzyme 6), then a GlcNAc is added by GlcNAcT-I (enzyme 2). Other than its natural acceptor Man<sub>5</sub>GlcNAc<sub>2</sub>-R, GlcNAcT-I can also recognize Man<sub>3</sub>GlcNAc<sub>2</sub>-R as its substrate and add one GlcNAc to the mannose core structure to form GlcNAcMan<sub>3</sub>GlcNAc<sub>2</sub>-peptide.

30 **Route III:** The  $\alpha$ 1,6-linked mannose is removed by an  $\alpha$ 1,6-mannosidase, followed by the addition of GlcNAc by GlcNAcT-I and removal of the terminal  $\alpha$ 1,3-linked mannose

by an  $\alpha$ 1,3-mannosidase. From the experimental data obtained, GlcNAcT-I can recognize this  $\text{Man}_4\text{GlcNAc}_2$ -peptide as acceptor and add one GlcNAc residue to form  $\text{GlcNAcMan}_4\text{GlcNAc}_2$ -peptide.

5 **Route IV:** Similar to Route III,  $\alpha$ 1,3-linked mannose is removed by an  $\alpha$ 1,3-mannosidase, followed by GlcNAcT-I reaction. Then the terminal  $\alpha$ 1,6-linked mannose can be removed by an  $\alpha$ 1,6-mannosidase.

10 After the function of GlcNAcT-I (responsible for the addition of the GlcNAc  $\beta$ 1,2-linked to the  $\alpha$ 1,3-mannose on the mannose core) and GlcNAcT-II (responsible for the addition of a second GlcNAc  $\beta$ 1,2-linked to the  $\alpha$ 1,6-mannose on the mannose core), the  $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ -peptide can be processed by GalT 1 and sialyltransferase to form bi-antennary complex N- Glycans. Other GlcNAc transferases such as GlcNAcT-IV, GlcNAcT-V, and/or GlcNAcT-VI (Figure 188 and Figure 189) can also glycosylate the  $\text{GlcNAc}_2\text{Man}_3\text{GlcNAc}_2$ -peptide. Additional glycosylation by the GalT 1 and sialyltransferases will form multi-antennary complex N-glycans. The enzyme GlcNAcT-III catalyzes the insertion of a bisecting GlcNAc, thus preventing the actions of ManII and 15 subsequent action of transferases GlcNAcT-II, GlcNAcT-IV and GlcNAcT-V.

#### Tissue-Type Plasminogen Activator (TPA)

##### 39. Fucosylation of TPA to create Sialyl Lewis X

20 This example sets forth the preparation of Tissue Tissue-type Plasminogen Activator (TPA) with N-linked sialyl Lewis X antigen.

25 **Sialylation.** TPA expressed in mammalian cells will often contain a majority of the glycans terminating in sialic acid, but to ensure complete sialylation, it would be beneficial to first perform an *in vitro* sialylation. TPA in a suitable buffer (most preferably between pH 5.5 and 9, for example Tris buffered saline, pH 7.2) is incubated with CMP sialic acid and sialyltransferase for a time sufficient to convert any glycans lacking sialic acid to sialylated species. Typical conditions would be 1 mg/mL TPA, 3 mM CMP sialic acid, 0.02 U/mL ST3Gal3, 32°C for 24 hours. Microbial growth can be halted either by sterile filtration or the inclusion of 0.02% sodium azide. The TPA concentration is most preferably in the range 0.1 mg/mL up to the solubility limit of the peptide. The concentration of CMP-SA should be 30

sufficient for there to be excess over the available sites, and might range from 50  $\mu$ M up to 50 mM, and the temperature from 2°C up to 40°C. The time required for complete reaction will depend on the temperature, the relative amounts of enzyme to acceptor substrate, the donor substrate concentration, and the pH. Other sialyltransferases that may be capable of adding 5 sialic acid in 2,3 linkage include ST3Gal4; microbial transferases could also be used.

**Fucosylation.** Typical conditions for fucosylation would be 1 mg/mL TPA, 3 mM GDP-fucose, 0.02 U/mL FTVI, 5 mM MnCl<sub>2</sub>, 32°C for 24H in Tris buffered saline.

Microbial growth can be halted either by sterile filtration or the inclusion of 0.02% sodium azide. The TPA concentration is most preferably in the range 0.1 mg/mL up to the solubility 10 limit of the peptide. The concentration of GDP-fucose should be sufficient for there to be excess over the available sites, and might range from 50  $\mu$ M up to 50 mM, and the temperature from 2°C up to 40°C. The time required for complete reaction will depend on the temperature, the relative amounts of enzyme to acceptor substrate, the donor substrate concentration, and the pH. Other fucosyltransferases that may be capable of making sialyl 15 Lewis x include FTVII, FTV, FTIII, as well as microbial transferases could also be used.

40. Trimming of high mannose to tri-mannose core structure: Tissue-type Plasminogen Activator produced in CHO

This example sets forth the preparation of Tissue-type Plasminogen Activator with a 20 trimannose core by trimming back from a high mannose glycan.

Tissue-type plasminogen activator (TPA) is currently produced in Chinese Hamster Ovary (CHO) cells and contains a low amount of high mannose N-linked oligosaccharide. The mannoses can be trimmed down using a variety of the specific mannosidases. The first step is to generate Man5GlcNAc2(Fuc0-1) from Man9GlcNAc2(Fuc0-1). This can be done 25 using mannosidase I. Then either GlcNAcT1 (GlcNAc transferase I) is used to make GlcNAc1Man5GlcNAc2(Fuc0-1) or Mannosidase III is used to make Man3GlcNAc2(Fuc0-1). From Man3GlcNAc2(Fuc0-1), GlcNAc1Man3GlcNAc2(Fuc0-1) can be produced using 30 GlcNAcT1 or from GlcNAc1Man5GlcNAc2(Fuc0-1), GlcNAc1Man3GlcNAc2(Fuc0-1) can be produced using Mannosidase II. GlcNAc1Man3GlcNAc2(Fuc0-1) is then converted into GlcNAc2Man3GlcNAc2(Fuc0-1) using GlcNAcTransferase II (GlcNAcTII). The two

terminal GlcNAc residues are then galactosylated using GalTII and then sialylated with SA-PEG using ST3GalIII.

Conversely, TPA can be produced in yeast or fungal systems. Similar processing would be required for fungal derived material.

5

41. Generation and PEGylation of GlcNAc-ASN structures: TPA produced in Yeast

This example sets forth the preparation of PEGylated GlcNAc-Asn structures on a peptide such as TPA expressed in yeast.

10 Yeast expression is expected to result in a TPA which contains a single N-linked mannan-type structure. This recombinant glycoprotein is first treated with endoglycosidase H to generate GlcNAc structures on the asparagine (Asn) residues on the peptide.

15 The GlcNAc-Asn structures on the peptide/protein backbone are then modified with galactose or galactose-PEG using UDP-galactose or UDP-galactose-6-PEG, respectively, and a galactosyltransferase such as GalT1. In one case, the galactose-PEG is the terminal residue. In the second case, the galactose is further modified with SA-PEG using a CMP-SA-PEG donor and a sialyltransferase such as ST3GalIII. In another embodiment, the GlcNAc-Asn structures on the peptide/protein backbone may be galactosylated and sialylated as described above, and then further sialylated using CMP-SA-20 PEG and an  $\alpha$ 2,8-sialyltransferase such as the enzyme encoded by the *Campylobacter jejuni* cst-II gene.

Transferrin

42. GlycoPEGylation of Transferrin

25 This example sets forth the preparation of asialotransferrin and its sialylation with PEG-CMP-sialic acid.

30 **Preparation of Asialo-transferrin.** Human-derived holo-Transferrin, (10 mg) was dissolved in 500  $\mu$ L of 50 mM NaOAc, 5 mM CaCl<sub>2</sub>, pH 5.5. To this solution was added 500 mU Neuraminidase II (*Vibrio cholerae*) and the reaction mixture was shaken gently for 20.5 hours at 37 °C. The reaction mixture was added to the prewashed N-(*p*-aminophenyl)oxamic acid-agarose conjugate (600  $\mu$ L) and the washed beads gently rotated

for 24 hours at 4 °C. The mixture was centrifuged at 10,000 rpm and the supernatant was collected. The reaction mixture was adjusted to 5 mM EDTA by addition of 100  $\mu$ L of 30 mM EDTA to the washed beads, which were gently rotated for 20 hours at 4 °C. The suspension was centrifuged for 2 minutes at 10,000 rpm and the supernatant was collected.

5 The beads were washed 5 times with 0.35 mL of 50 mM NaOAc, 5 mM CaCl<sub>2</sub>, 5 mM EDTA, pH 5.5 and all supernatants were pooled. The enzyme solution was dialyzed twice at 4 °C into 15 mM Tris-HCl, 1 M NaCl, pH 7.4. 0.3 mL of the transferrin solution (3.3 mL total) was removed and dialyzed twice against water. The remainder was dialyzed twice more at 4 °C against phosphate buffered saline. The dialyzed solution was stored at -20 °C.

10 Protein samples were analyzed by IEF Electrophoresis. Samples (9  $\mu$ L, 25  $\mu$ g) were diluted with 16  $\mu$ L Tris buffer and mixed with 25  $\mu$ L of the sample loading buffer and applied to Isoelectric Focusing Gels (pH 3-7). Gels were run and fixed using standard procedures. Gels were stained with Colloidal Blue Stain.

**Sialyl-PEGylation of asialo-Transferrin.** Desialylated transferrin (250  $\mu$ g) and 15 CMP-sialic acid or CMP-SA-PEG (1 kDa or 10 kDa)(0.05  $\mu$ mol) were dissolved in 69  $\mu$ L 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN<sub>3</sub>, pH 7.2 in 1.5 mL plastic tubes. The tubes were vortexed briefly and 100 mU ST3Gal3 (90  $\mu$ L) were added (total volume 250  $\mu$ L). The tubes were vortexed again and mixed gently for 24 hours at 32 °C. The reactions were stopped by freezing at -80 °C. Novex Tris-Glycine 8-16% 1 mm gels were used for SDS PAGE analysis (Figure 190). Samples (2.5  $\mu$ L, 25  $\mu$ g) were mixed with 25  $\mu$ L of sample loading buffer and 0.4  $\mu$ L of  $\beta$ -mercaptoethanol and heated for 6 minutes at 85 °C. Gels were run using standard conditions and stained with Colloidal Blue Stain. IEF gels were also performed as described above (Figure 191). Samples were also dialyzed against water analyzed by MALDI-TOF.

25 **Results.** MALDI was also performed. Native transferrin (78729); asialotransferrin (78197); resialylated transferrin (79626/80703); with SA-PEG 1k (79037 (1); 80961 (2); 82535 (3); 84778 (4)); with SA-PEG 5k (90003 (2); 96117 (3); 96117 (4)); with SA-PEG 10k (100336 (2); 111421 (3); 122510 (4)).

### 43. Transferrin-GDNF

This example sets forth the procedures for the glycoconjugation of proteins, and in particular, transferrin is glycoconjugated to GDNF. Transferrin-SA-Linker-Gal-UDP is prepared from transferrin. The galactose residue is removed from GDNF glycans, and

5 Transferrin-SA-Linker-Gal-UDP is conjugated to GDNF glycans using a galactosyltransferase.

**Preparation of agalacto-GDNF.** GDNF produced in NSO cells (NSO murine myeloma cells) is dissolved at 2.5 mg/mL in 50 mM Tris 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, and is incubated with 300 mU/mL beta-galactosidase-agarose conjugate for 16 hours at 10 32°C. To monitor the reaction a small aliquot of the reaction is diluted with the appropriate buffer and a IEF gel performed according to Invitrogen procedures. The mixture is centrifuged at 10,000 rpm and the supernatant is collected. The supernatant is dialyzed at 4 °C against 50 mM Tris -HCl pH 7.4, 1 M NaCl, 0.05% NaN<sub>3</sub> and then twice more against 50 mM Tris -HCl pH 7.4, 1 M NaCl, 0.05% NaN<sub>3</sub>. The dialyzed solution is then concentrated 15 using a Centricon Plus 20 centrifugal filter and stored at -20 °C. The conditions for the IEF gel are run according to the procedures and reagents provided by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

**Preparation of Transferrin-SA-Linker-Gal-UDP.** Asialo-transferrin is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is 20 incubated with CMP-sialic acid-linker-Gal-UDP (molar amount to add 1 molar equivalent of nucleotide sugar to transferrin) and 0.1 U/mL of ST3Gal3 at 32°C for 2 days. To monitor the incorporation of sialic acid, a small aliquot of the reaction has <sup>14</sup>C-SA-UDP ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The radioactive label 25 incorporation into the peptide is quantitated using an in-line radiation detector.

The solution is incubated with 5 mM CMP-sialic acid and 0.1 U/mL of ST3Gal3 (to cap any unreacted transferrin glycans) at 32°C for 2 days. The incorporation into the peptide is quantitated using an in-line UV detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting 30 fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE

and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

**Preparation of Transferrin-SA-Linker-Gal-GDNF.** The transferrin-SA-Linker-Gal-UDP prepared as described above is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 5 mM MnCl<sub>2</sub>, 0.05% NaN<sub>3</sub>, pH 7.2. The solution is incubated with 2.5 mg/mL agalacto-GDNF and 0.1 U/mL of galactosyltransferase at 32°C for 2 days. To monitor the incorporation of galactose, a small aliquot of the reaction has <sup>14</sup>C-galactose-UDP ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The radioactive label incorporation into the peptide is quantitated using an in-line radiation detector.

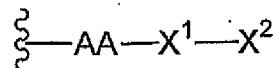
When the reaction is complete, the solution is incubated with 5 mM UDP-Gal and 0.1 U/mL of galactosyltransferase (to cap any unreacted transferrin glycans) at 32°C for 2 days followed by addition of 5 mM CMP-SA and 0.1 U/mL of ST3Gal3. After 2 additional days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

20 The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The 25 appended claims are intended to be construed to include all such embodiments and equivalent variations.

What is claimed:

1. A cell-free, in vitro method of remodeling a peptide comprising poly(ethylene glycol), the peptide having the formula:



5 wherein

AA is a terminal or internal amino acid residue of the peptide;

X<sup>1</sup>-X<sup>2</sup> is a saccharide covalently linked to the AA, wherein

X<sup>1</sup> is a first glycosyl residue; and

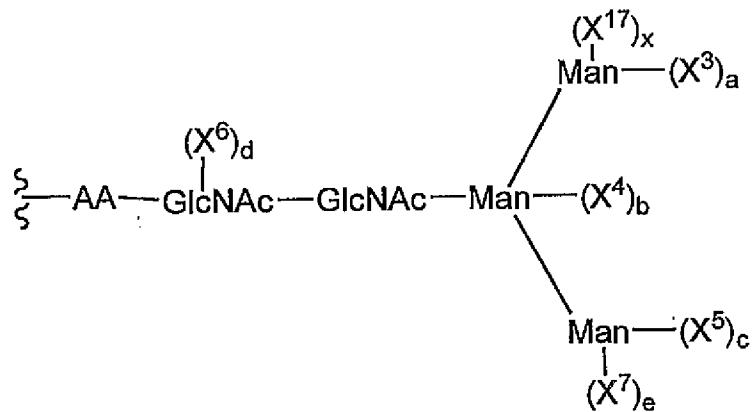
X<sup>2</sup> is a second glycosyl residue covalently linked to X<sup>1</sup>, wherein X<sup>1</sup> and X<sup>2</sup> are selected from monosaccharyl and oligosaccharyl residues;

10 the method comprising:

(a) removing X<sup>2</sup> or a saccharyl subunit thereof from the peptide, thereby forming a truncated glycan.

15 2. The method according to claim 1 wherein said truncated glycan is formed by removing a Sia residue.

3. The method according to claim 1 wherein said peptide has the formula:



20 wherein

$X^3, X^4, X^5, X^6, X^7$ , and  $X^{17}$ , are independently selected monosaccharyl or oligosaccharyl residues; and

a, b, c, d, e, and x are independently selected from the integers 0, 1 and 2.

5 4. The method according to claim 3 wherein said oligosaccharyl residue is a member selected from GlcNAc-Gal-Sia and GlcNAc-Gal.

10 5. The method according to claim 3 wherein at least one member selected from a, b, c, d, e and x is 1 or 2.

15 6. The method of claim 3, wherein said removing of step (a) produces a truncated glycan in which at least one of a, b, c, e and x are 0.

7. The method of claim 6, wherein  $X^3, X^5$  and  $X^7$  are members independently selected from  $(mannose)_z$  and  $(mannose)_z-(X^8)$

wherein

$X^8$  is a glycosyl moiety selected from mono- and oligo-saccharides; and z is an integer between 1 and 20, wherein

when z is 3 or greater, each  $(mannose)_z$  is independently selected from linear

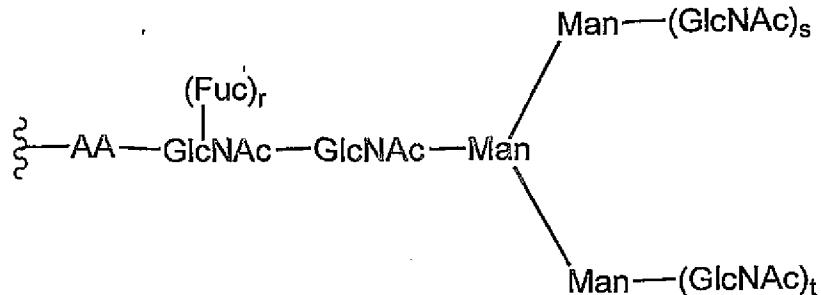
20 and branched structures.

8. The method of claim 6 wherein  $X^4$  is selected from the group consisting of GlcNAc and xylose.

25 9. The method of claim 6, wherein  $X^3, X^5$  and  $X^7$  are  $(mannose)_u$  wherein

u is selected from the integers between 1 and 20, and when u is 3 or greater, each  $(mannose)_u$  is independently selected from linear and branched structures.

30 10. The method according to claim 3 wherein said peptide has the formula:

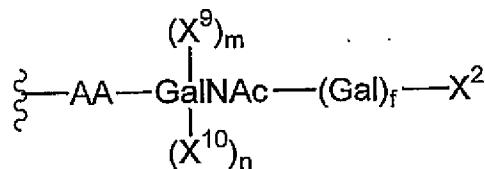


wherein

r, s, and t are integers independently selected from 0 and 1.

5

11. The method of claim 1, wherein said peptide has the formula:



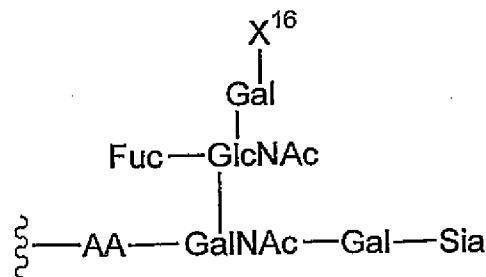
wherein

$\text{X}^9$  and  $\text{X}^{10}$  are independently selected monosaccharyl or oligosaccharyl

10 residues; and

$\text{m}$ ,  $\text{n}$  and  $\text{f}$  are integers independently selected from 0 and 1.

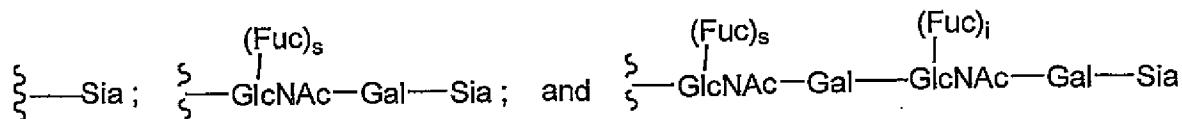
12. The method of claim 11, wherein said peptide has the formula:



15

wherein

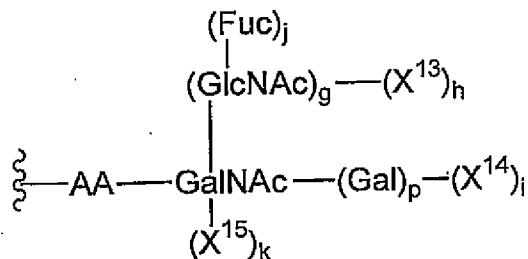
$\text{X}^{16}$  is a member selected from:



wherein

s and i are integers independently selected from 0 and 1.

5 13. The method of claim 12, wherein said peptide has the formula:



wherein

$X^{13}$ ,  $X^{14}$ , and  $X^{15}$  are independently selected glycosyl residues; and  
g, h, i, j, k, and p are independently selected from the integers 0 and 1

10

14. The method according to claim 13 wherein at least one of g, h, i, j, k  
and p is 1.

15

15. The method of claim 13, wherein

$X^{14}$  and  $X^{15}$  are members independently selected from GlcNAc and Sia; and  
i and k are independently selected from the integers 0 and 1.

20

16. The method according to claim 15 wherein at least one of i and k is 1,  
and if k is 1, g, h, and j are 0.

17. The method according to claim 1, further comprising:

(b) contacting the truncated glycan with at least one glycosyltransferase  
and at least one glycosyl donor under conditions suitable to transfer the at least one glycosyl

donor to the truncated glycan, thereby remodeling said peptide comprising poly(ethylene glycol).

18. The method according to claim 17 wherein said glycosyl donor  
5 comprises a modifying group covalently linked thereto.

19. The method of claim 1, further comprising:

(c) removing X<sup>1</sup>, thereby exposing AA.

10 20. The method according to claim 19, further comprising:  
(d) contacting AA with at least one glycosyltransferase and at least one glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to AA, thereby remodeling said peptide comprising poly(ethylene glycol).

15 21. The method according to claim 20 wherein said at least one glycosyl donor comprises a modifying group covalently linked thereto.

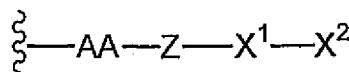
22. The method according to claim 21 wherein said modifying group is poly(ethylene glycol).

20 23. The method according to claim 22 wherein said poly(ethylene glycol) has a molecular weight distribution that is essentially homodisperse.

25 24. The method of claim 17, further comprising:  
(e) prior to step (b), removing a group added to said saccharide during post-translational modification.

25. The method of claim 24 wherein said group is a member selected from phosphate, sulfate, carboxylate and esters thereof.

30 26. The method of claim 1 wherein said peptide has the formula:

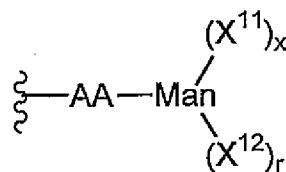


wherein

Z is a member selected from O, S, NH and a cross-linker.

5

27. The method of claim 1, wherein said peptide has the formula:



wherein

X<sup>11</sup> and X<sup>12</sup> are independently selected glycosyl moieties; and

r and x are integers independently selected from 0 and 1.

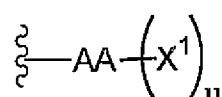
10

28. The method of claim 27, wherein X<sup>11</sup> and X<sup>12</sup> are (mannose)<sub>q</sub>, wherein q is selected from the integers between 1 and 20, and when q is three or greater, (mannose)<sub>q</sub> is selected from linear and branched structures.

15

29. A pharmaceutical composition comprising a pharmaceutically acceptable diluent and a remodeled peptide according to claim 1.

30. A cell-free, in vitro method of remodeling a peptide comprising poly(ethylene glycol), said peptide having the formula:



20

wherein

AA is a terminal or internal amino acid residue of said peptide;  
X<sup>1</sup> is a glycosyl residue covalently linked to said AA, selected from  
monosaccharyl and oligosaccharyl residues; and  
u is an integer selected from 0 and 1,

5        said method comprising:

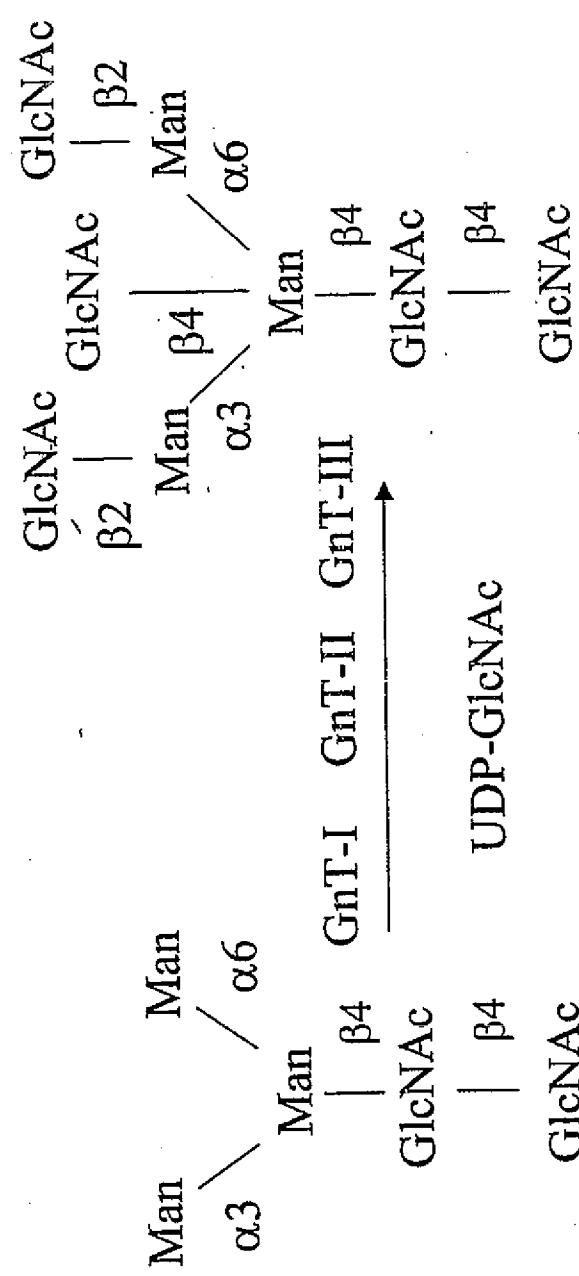
      contacting said peptide with at least one glycosyltransferase and at least one  
      glycosyl donor under conditions suitable to transfer said at least one glycosyl donor to said  
      truncated glycan, thereby remodeling said peptide.

10        31.        The method according to claim 30 wherein said at least one glycosyl  
      donor comprises a modifying group covalently linked thereto.

      32.        The method according to claim 30 wherein said modifying group is  
      poly(ethylene glycol).

15        33.        The method according to claim 32 wherein said poly(ethylene glycol)  
      has a molecular weight distribution that is essentially homodisperse.

20        34.        A pharmaceutical composition comprising a pharmaceutically  
      acceptable diluent and a remodeled peptide according to claim 30.



Trimannosyl core with  
Bisectioning GlcNAc

FIG. 1

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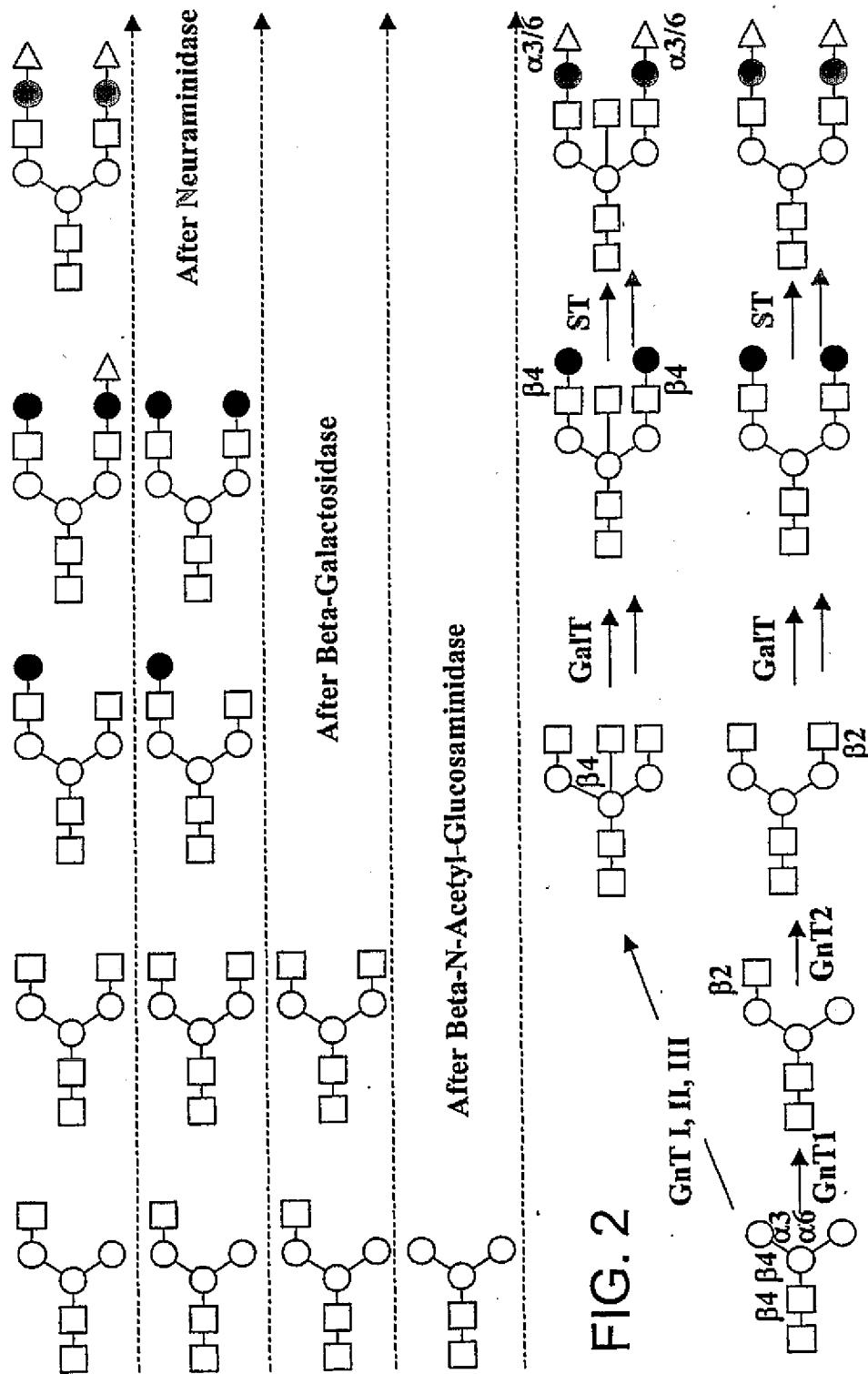
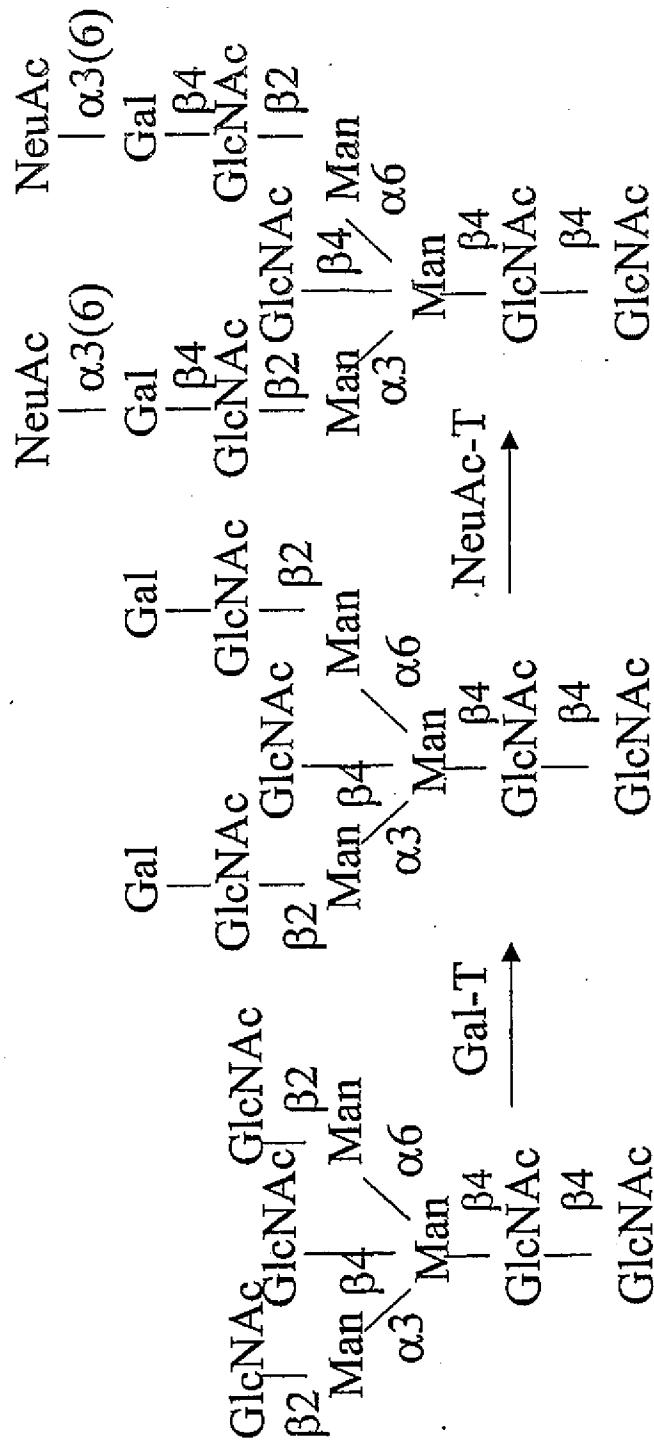


FIG. 2

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三  
五  
正

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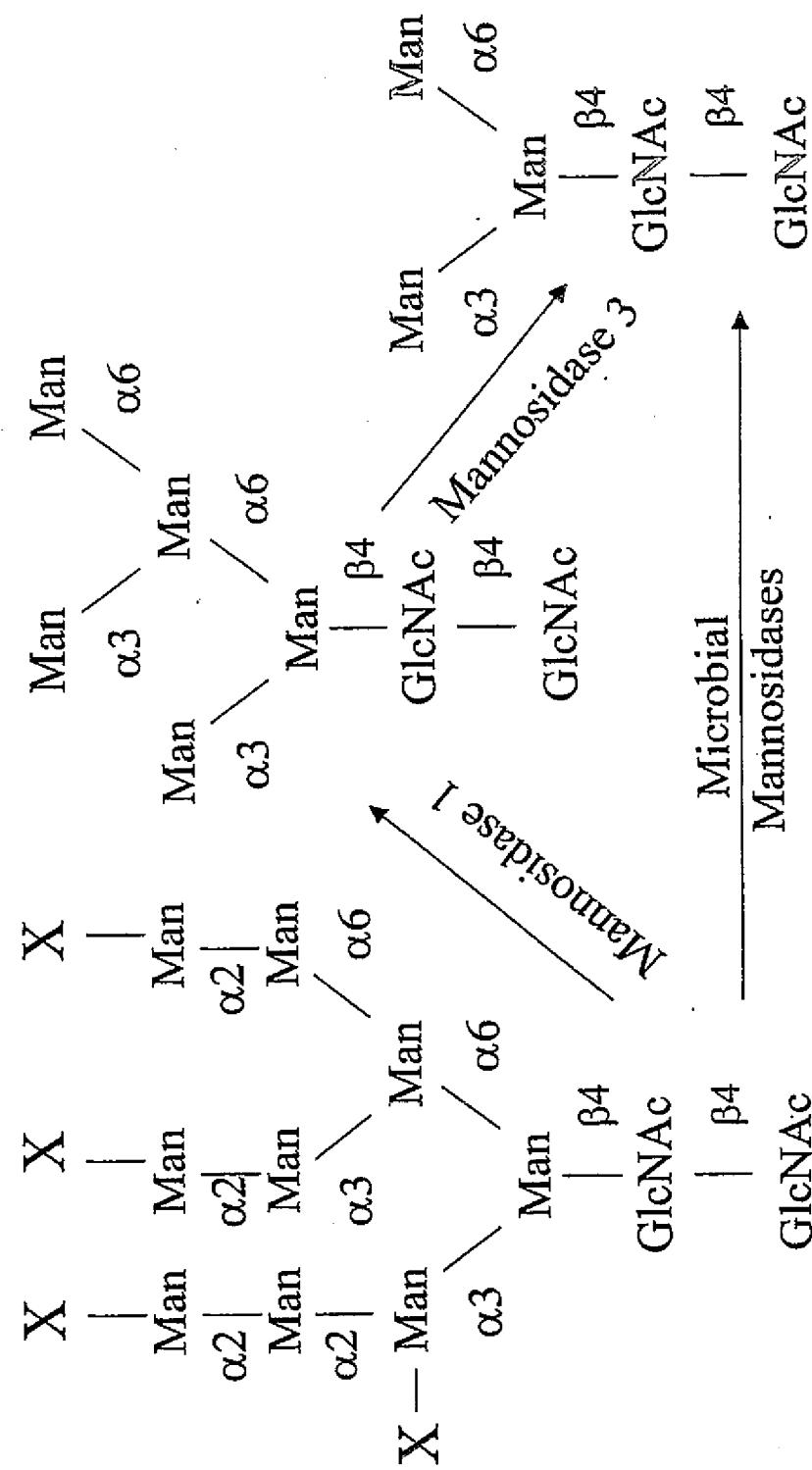
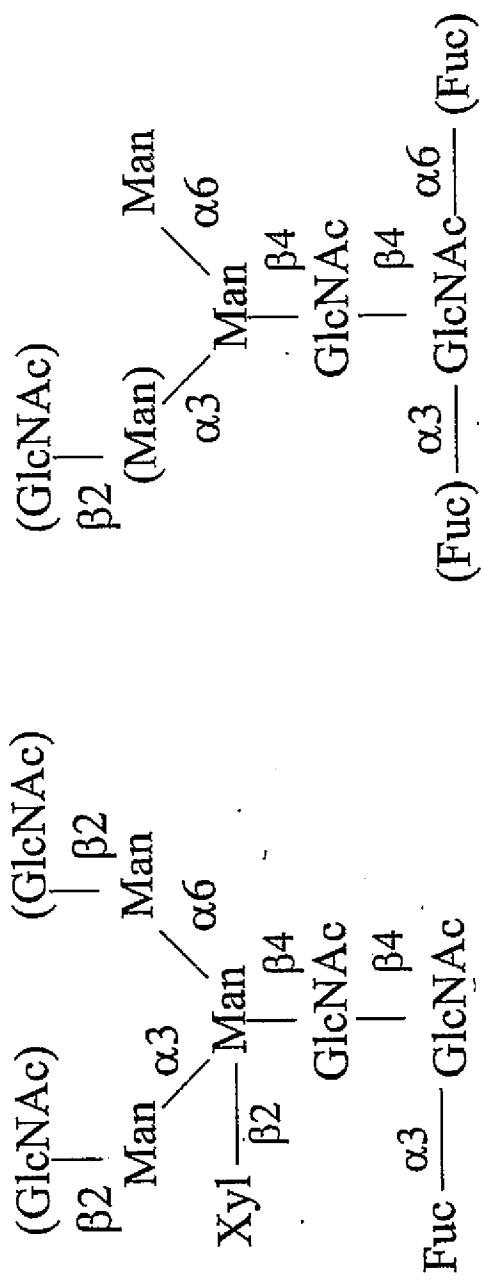


FIG. 4

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6  
FIG.

50

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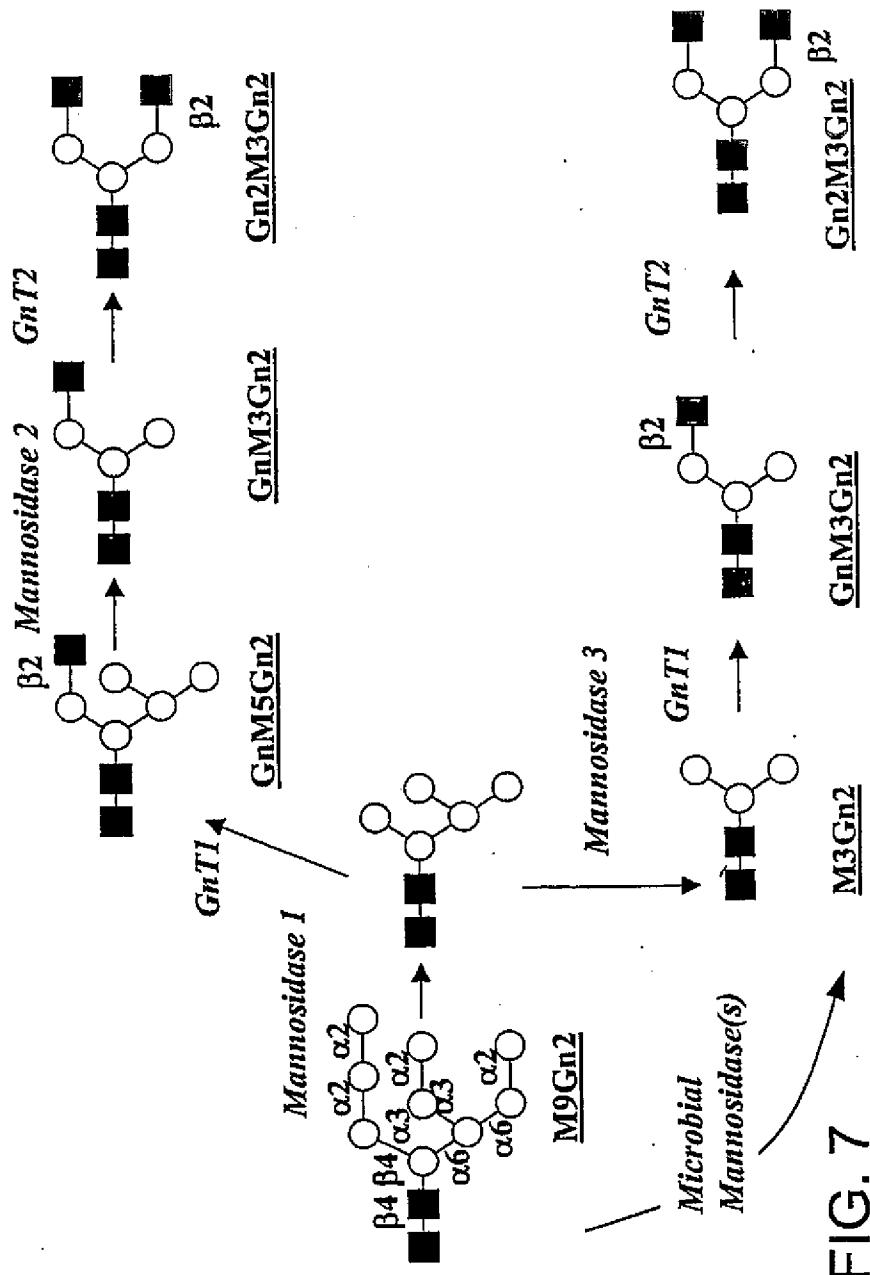
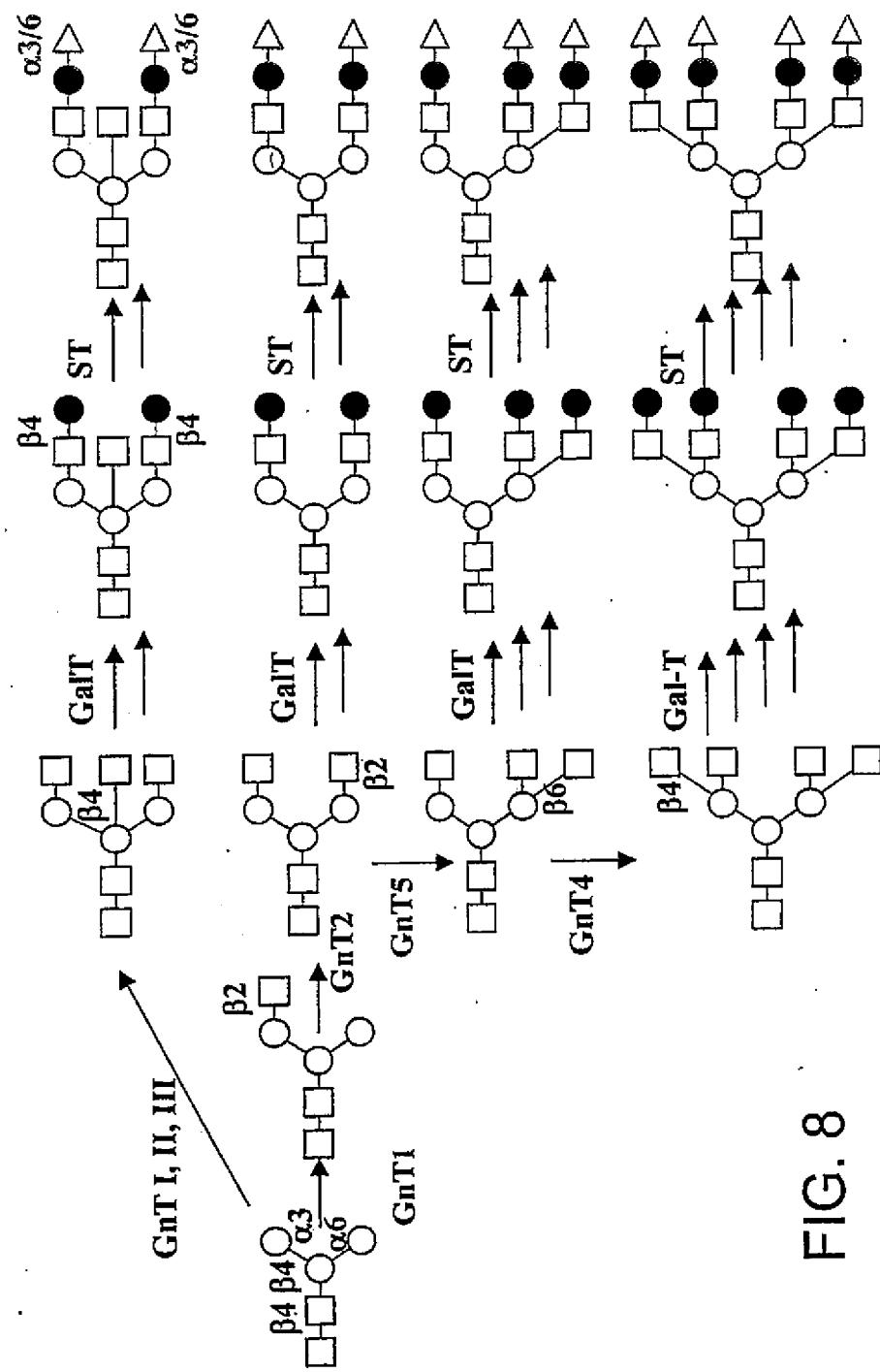


FIG. 7

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8  
FIG.

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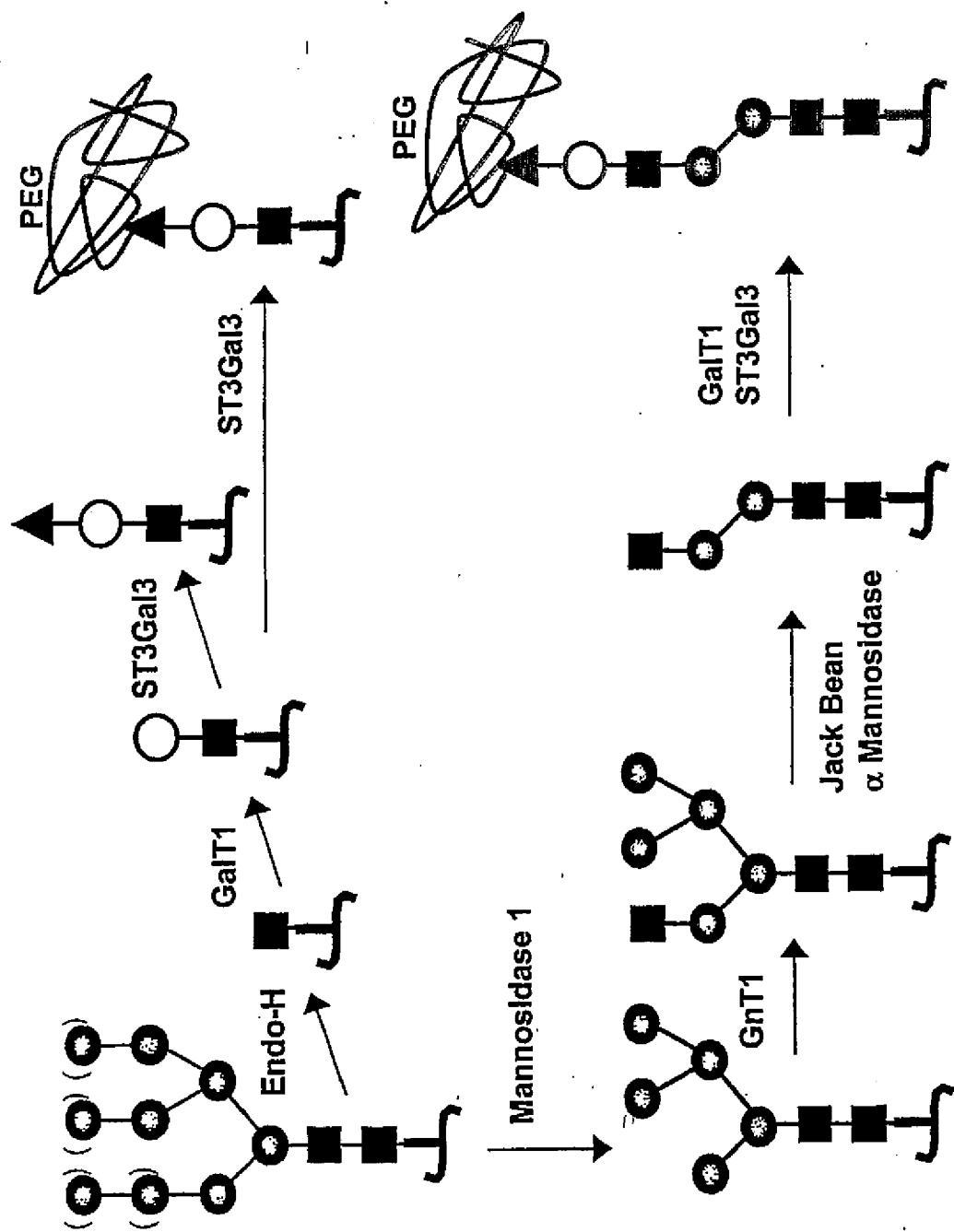


FIG. 9

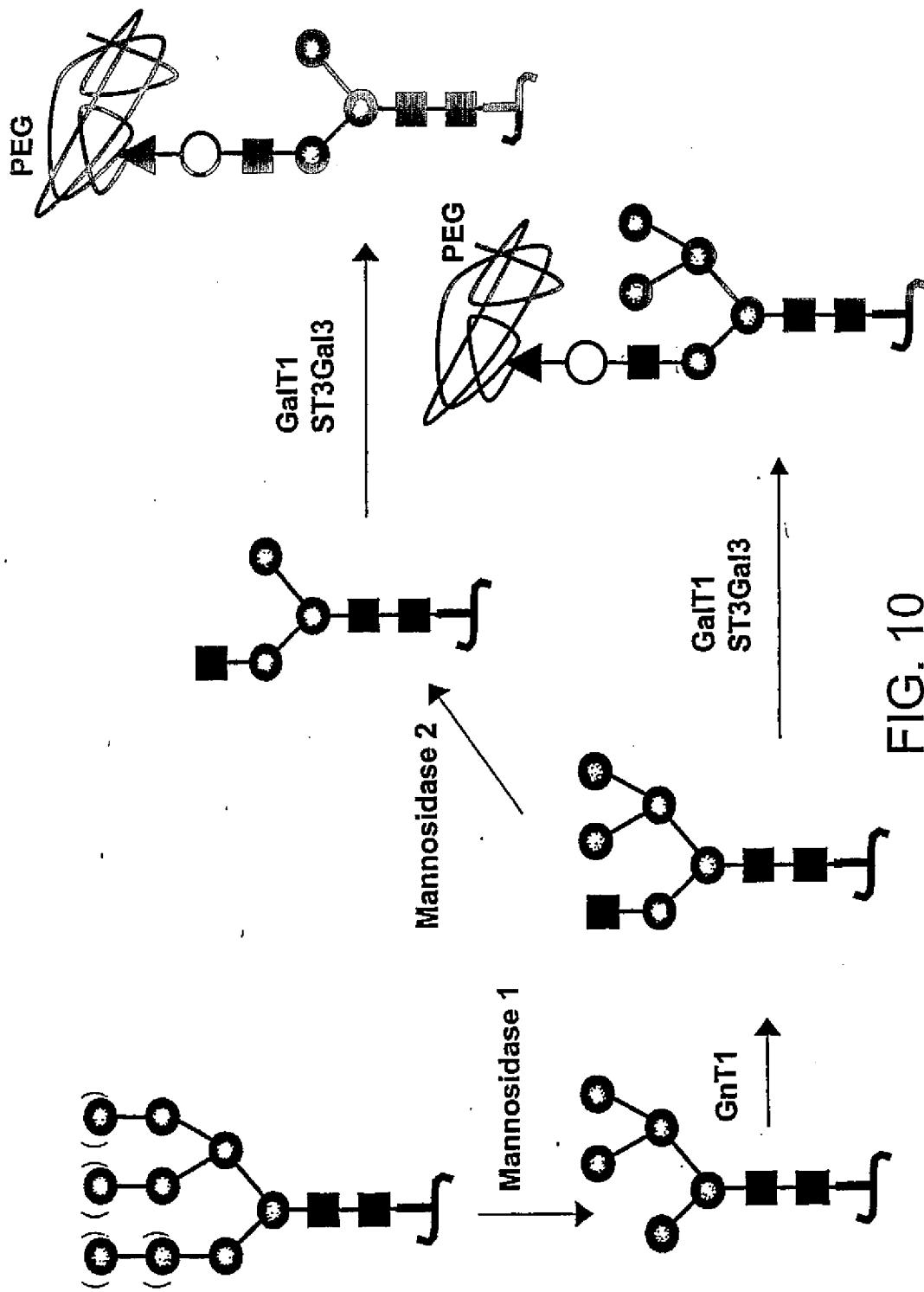


FIG. 10

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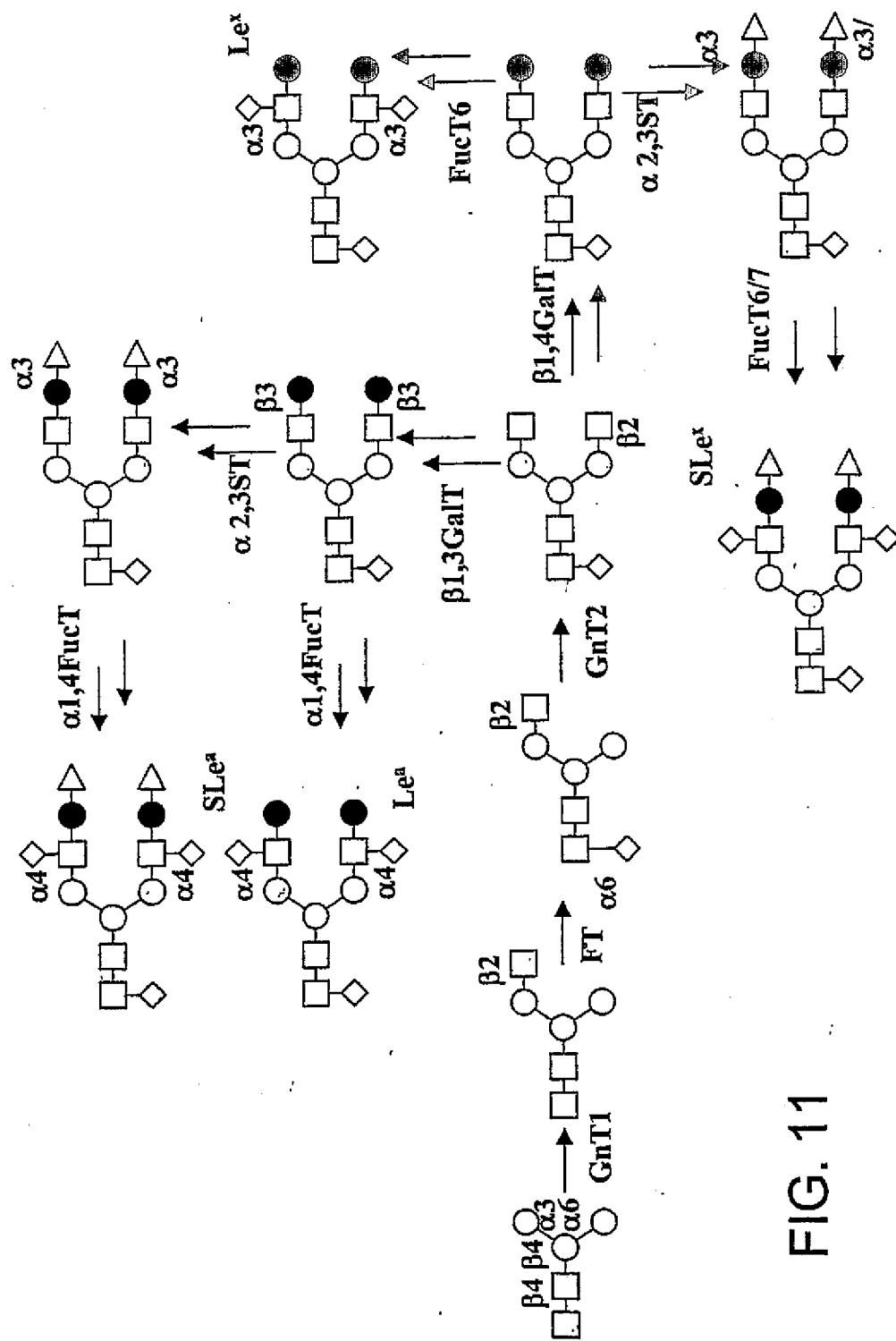


FIG. 11

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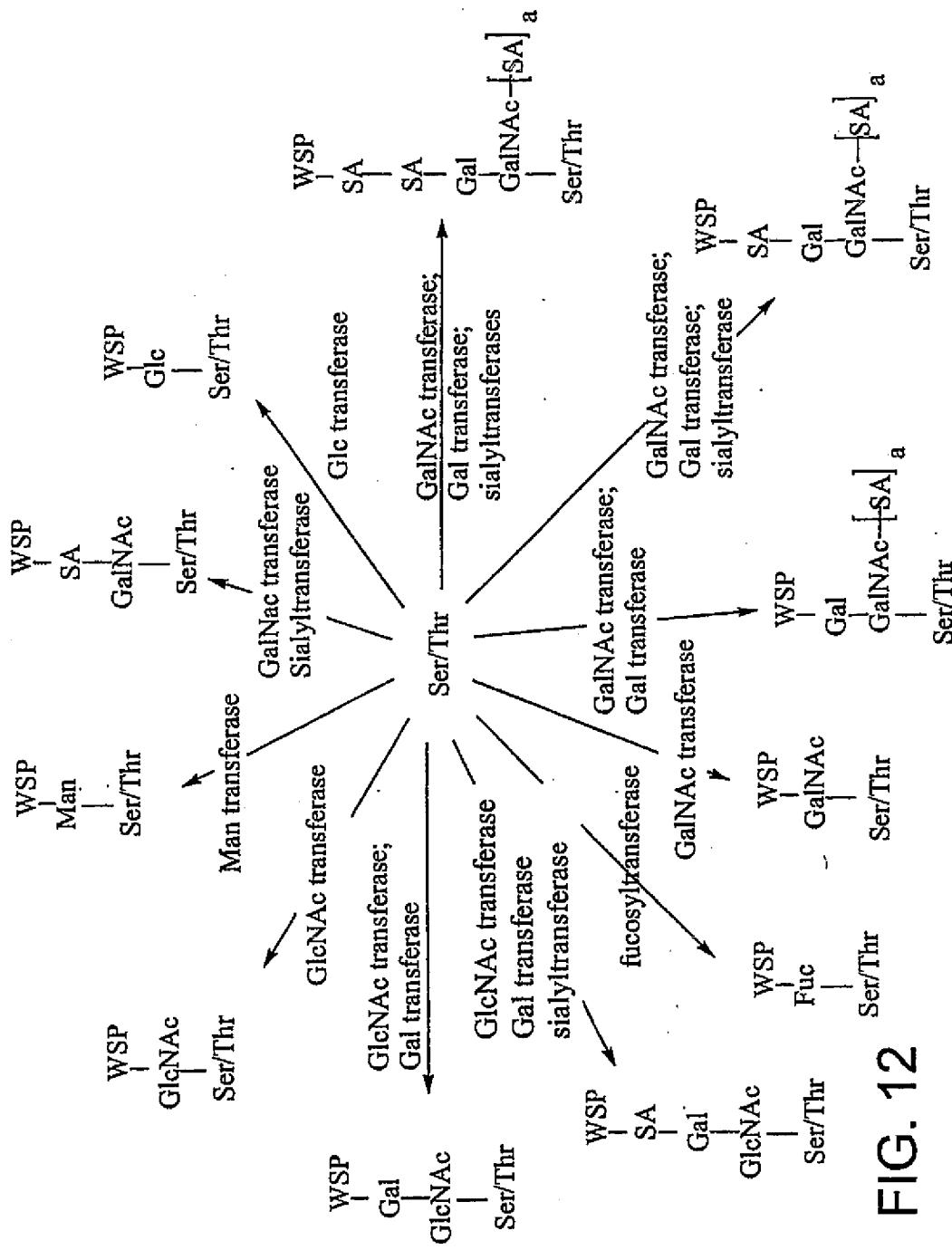


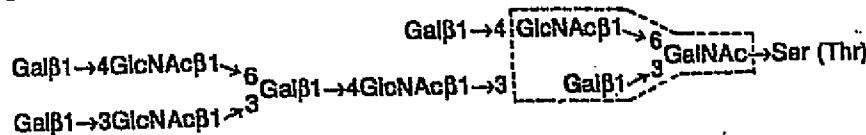
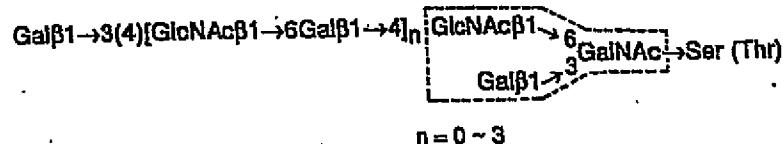
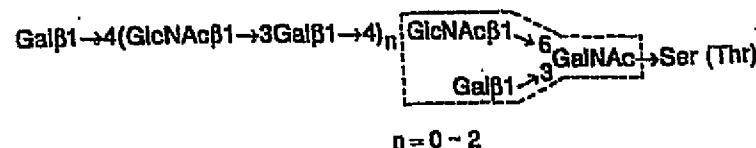
FIG. 12

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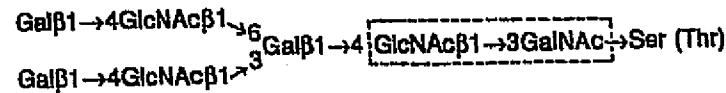
## Core 1



## Core 2



## Core 3

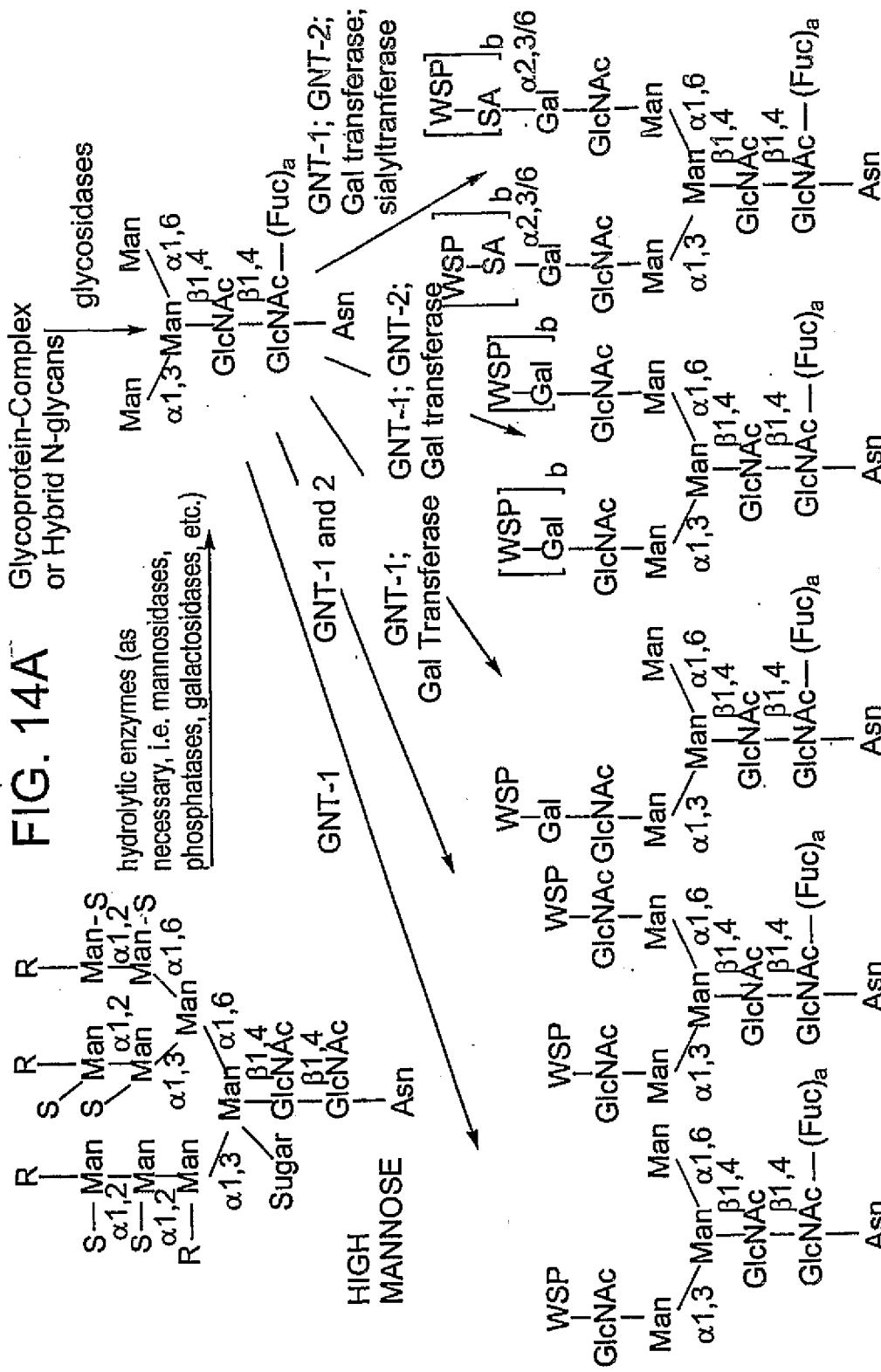


## Core 4



FIG. 13

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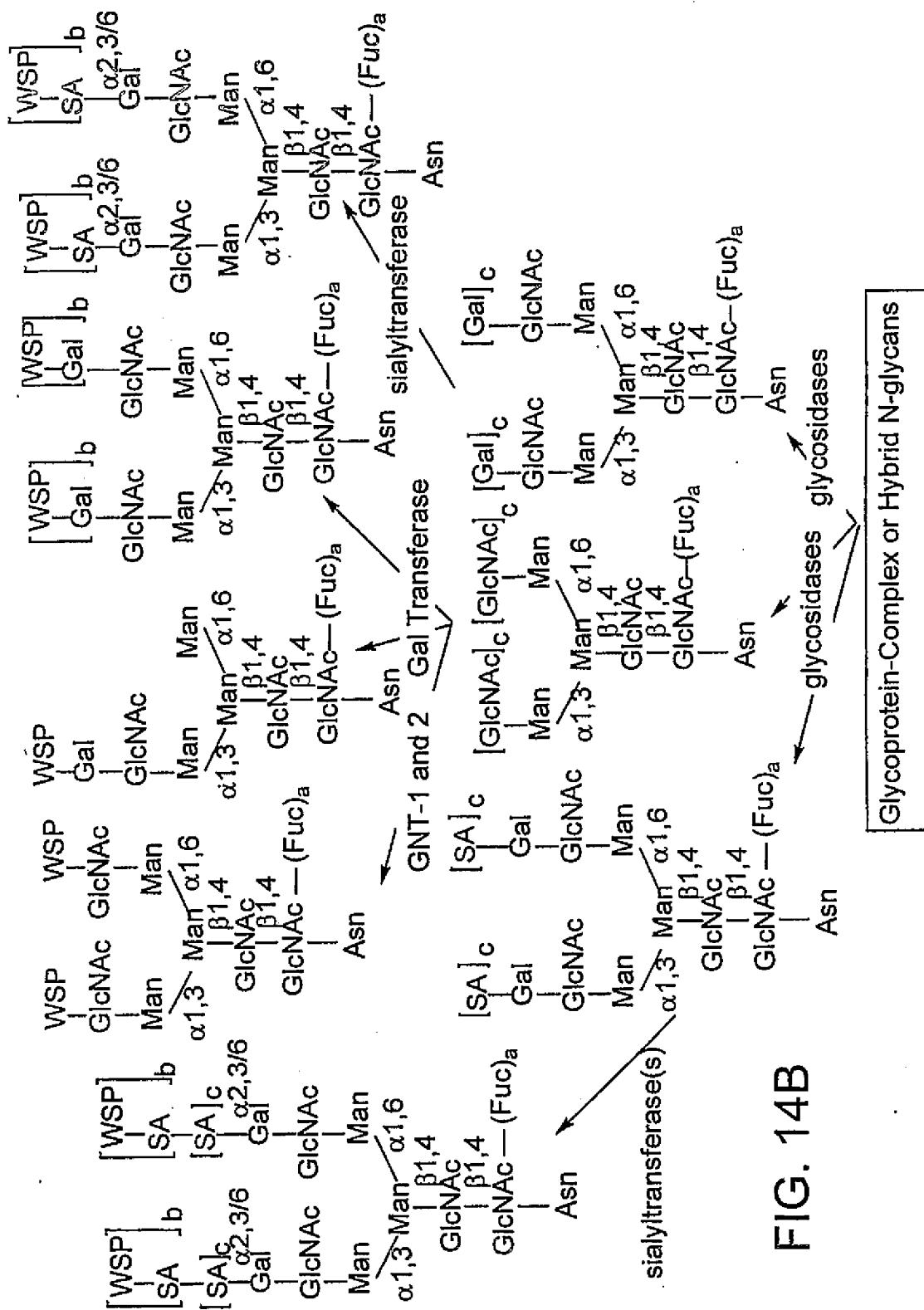


FIG. 14B

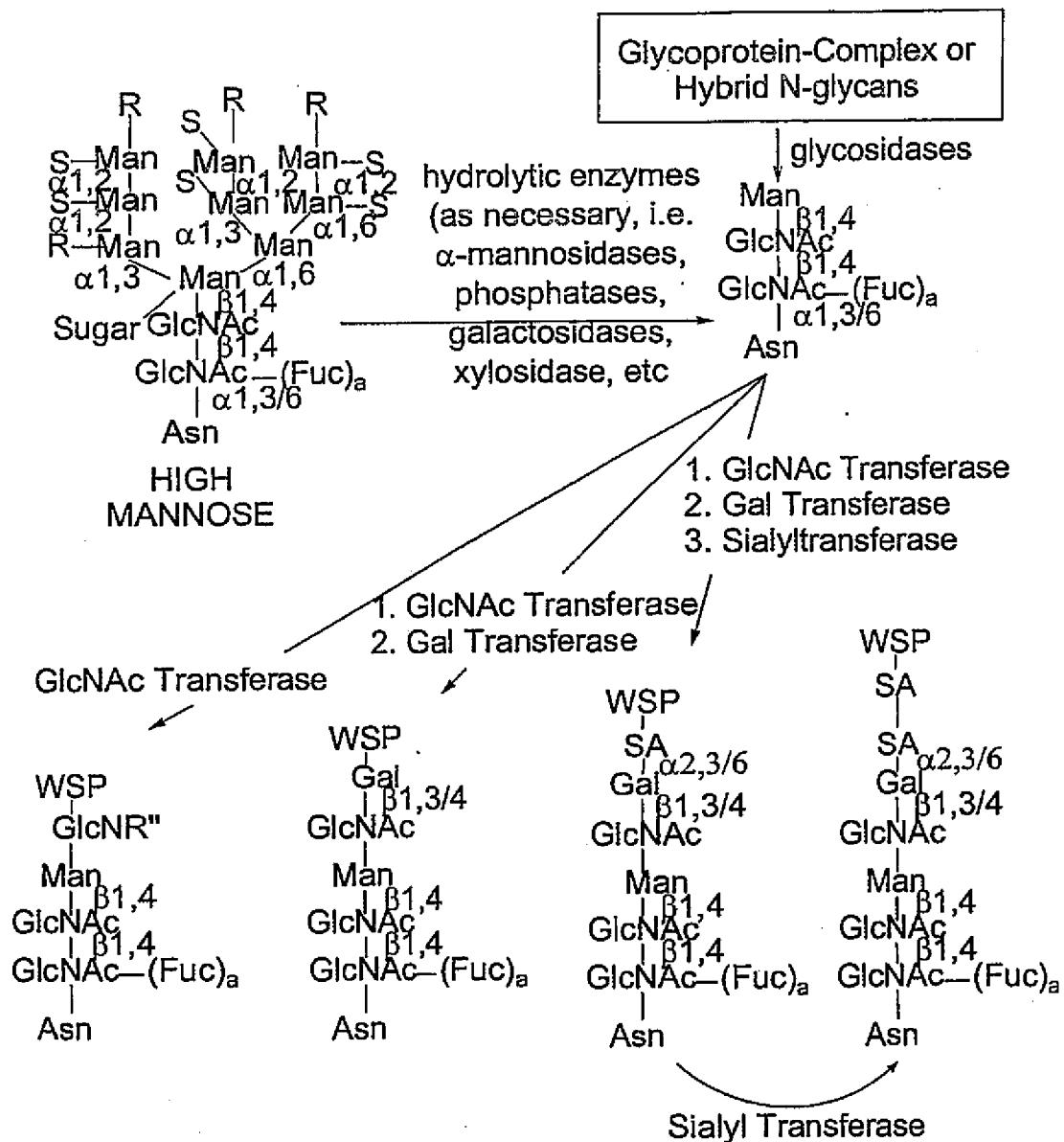


FIG. 15

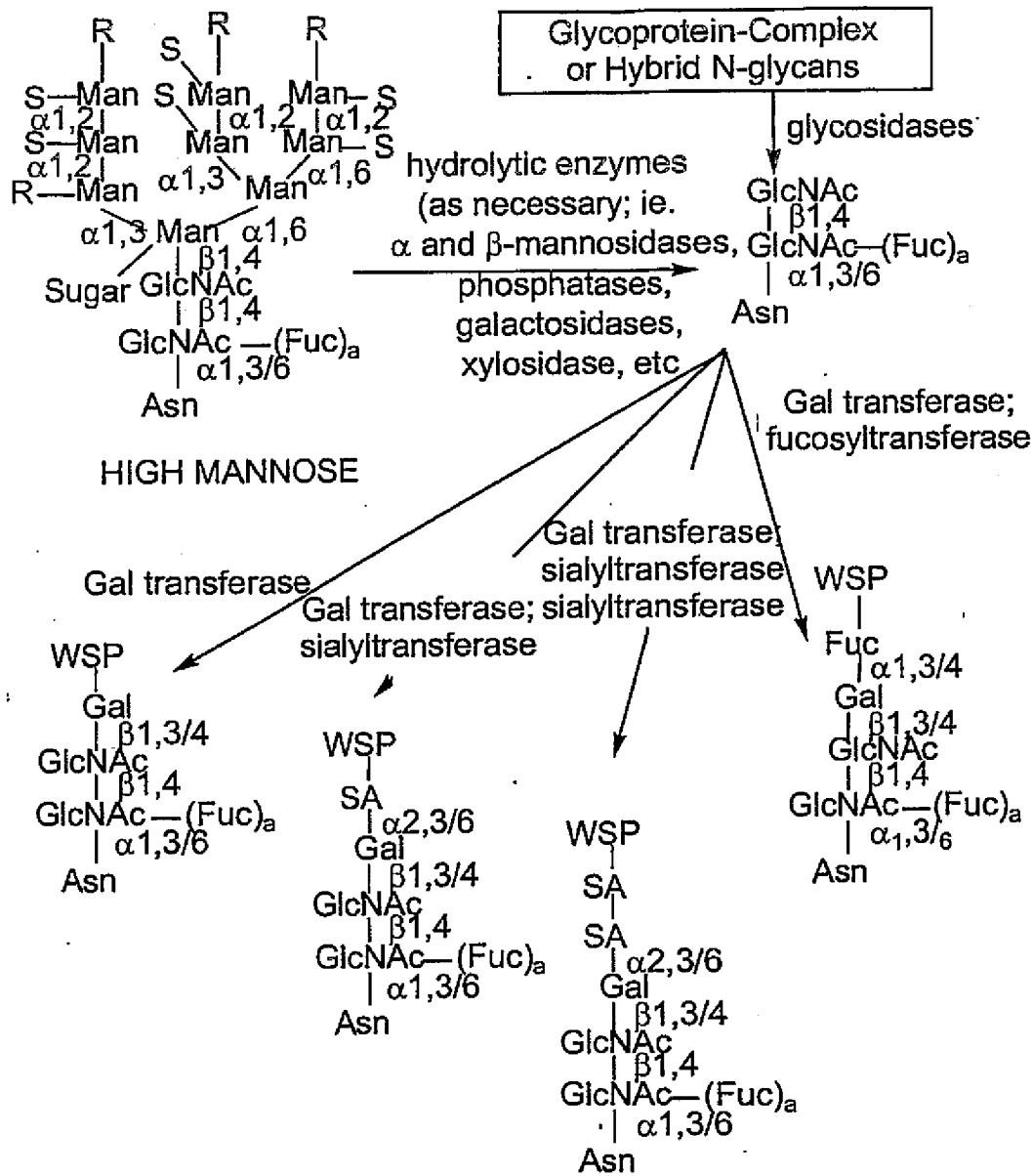


FIG. 16

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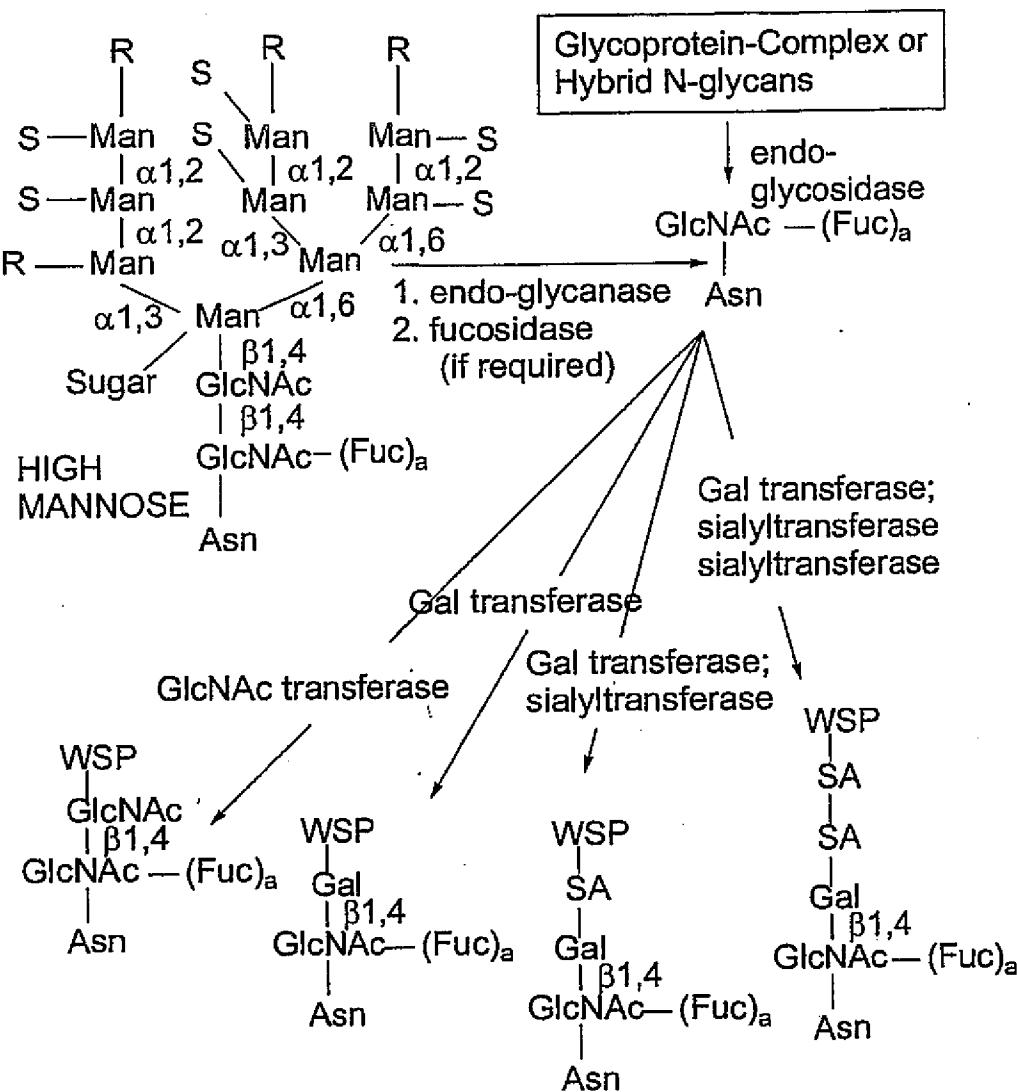
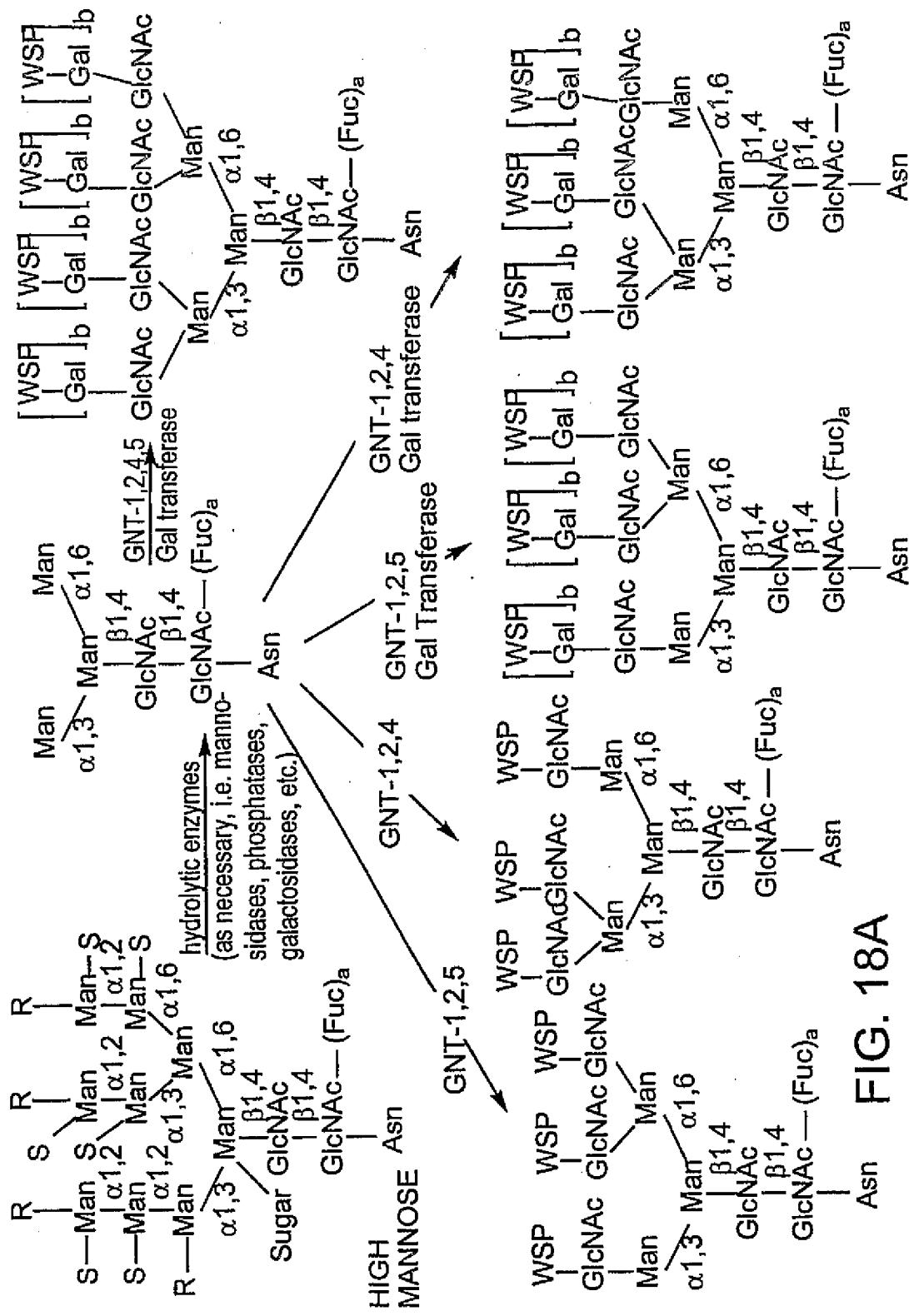


FIG. 17

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Asn FIG. 18A

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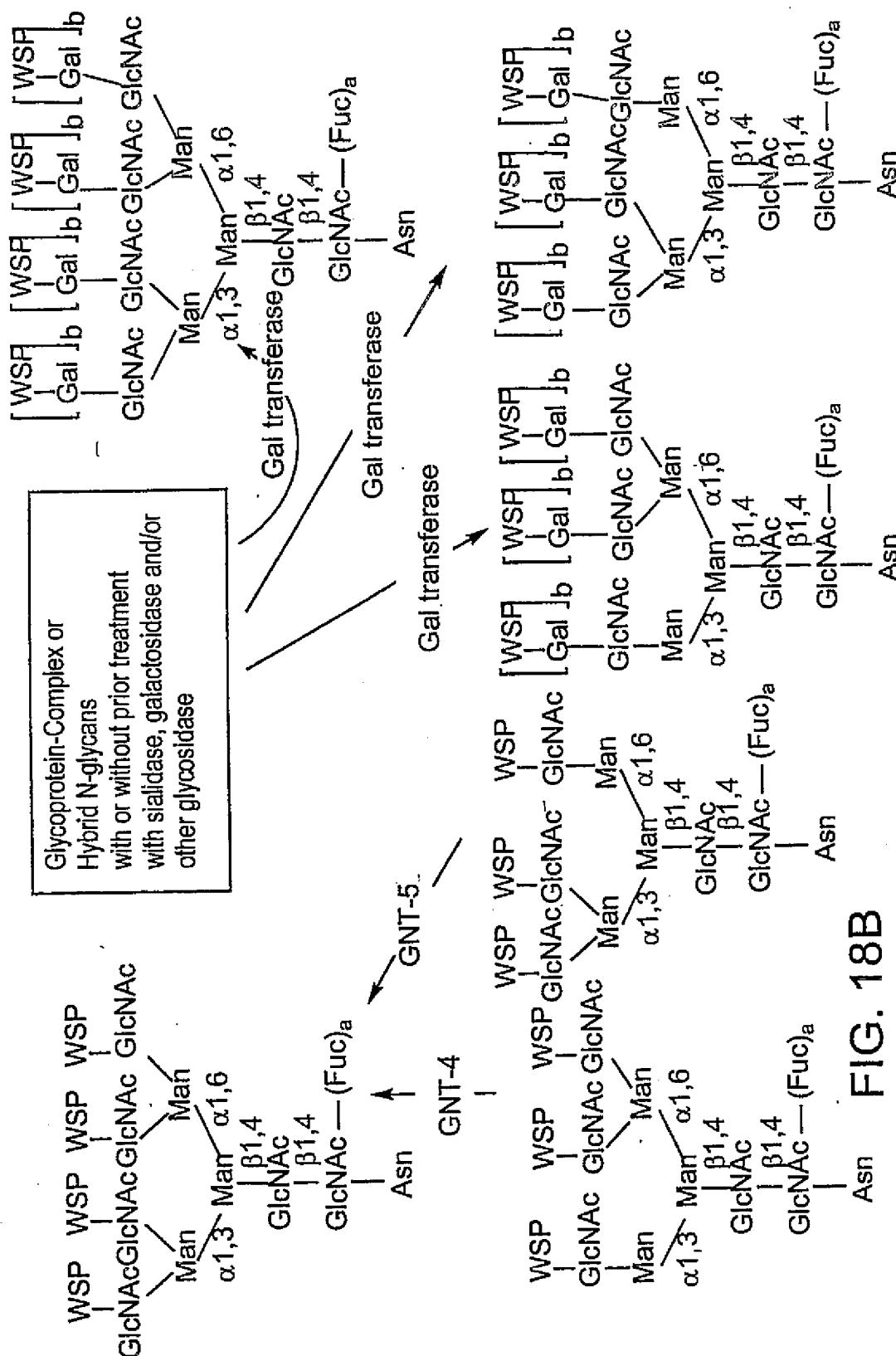
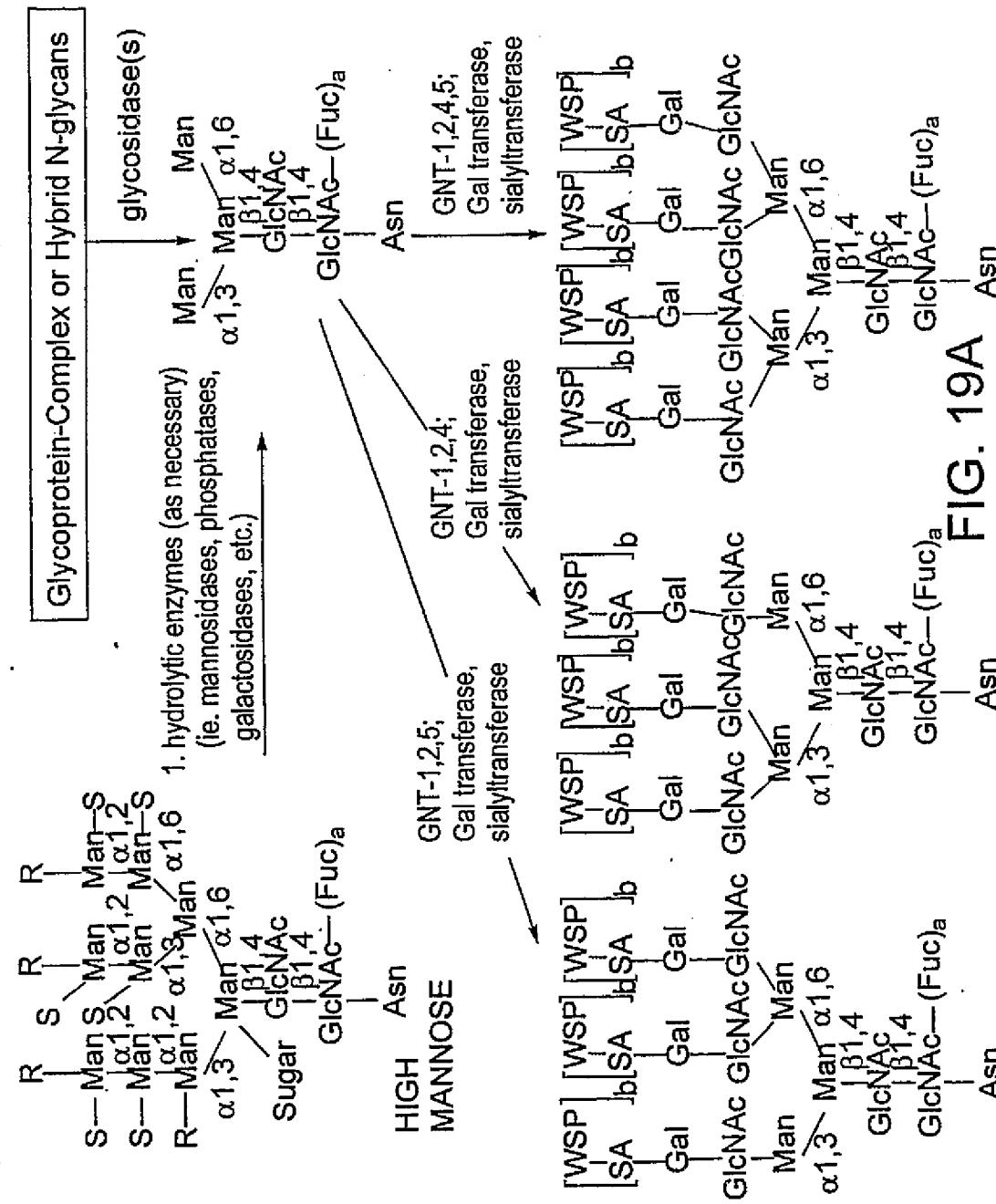
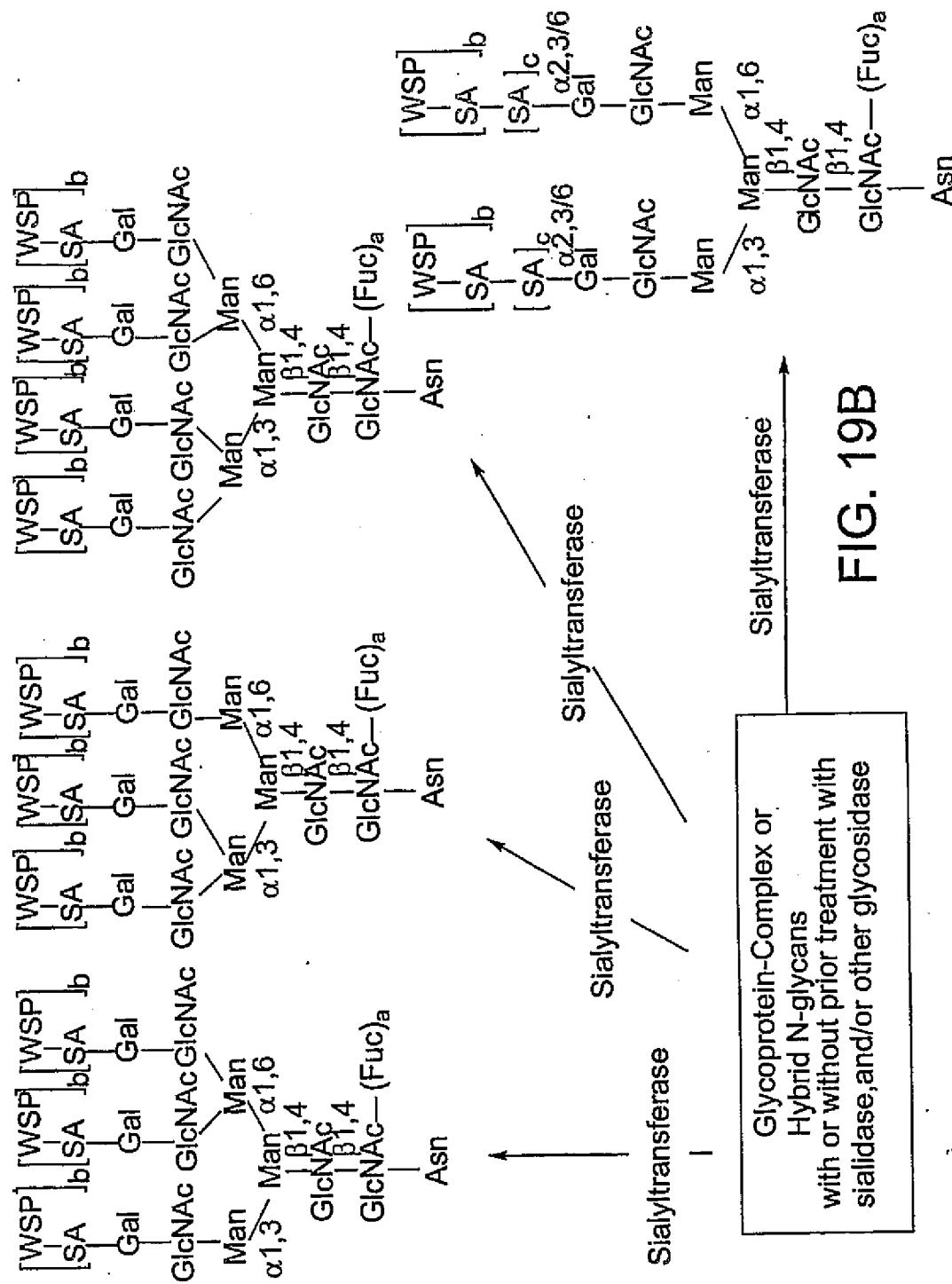


FIG. 18B



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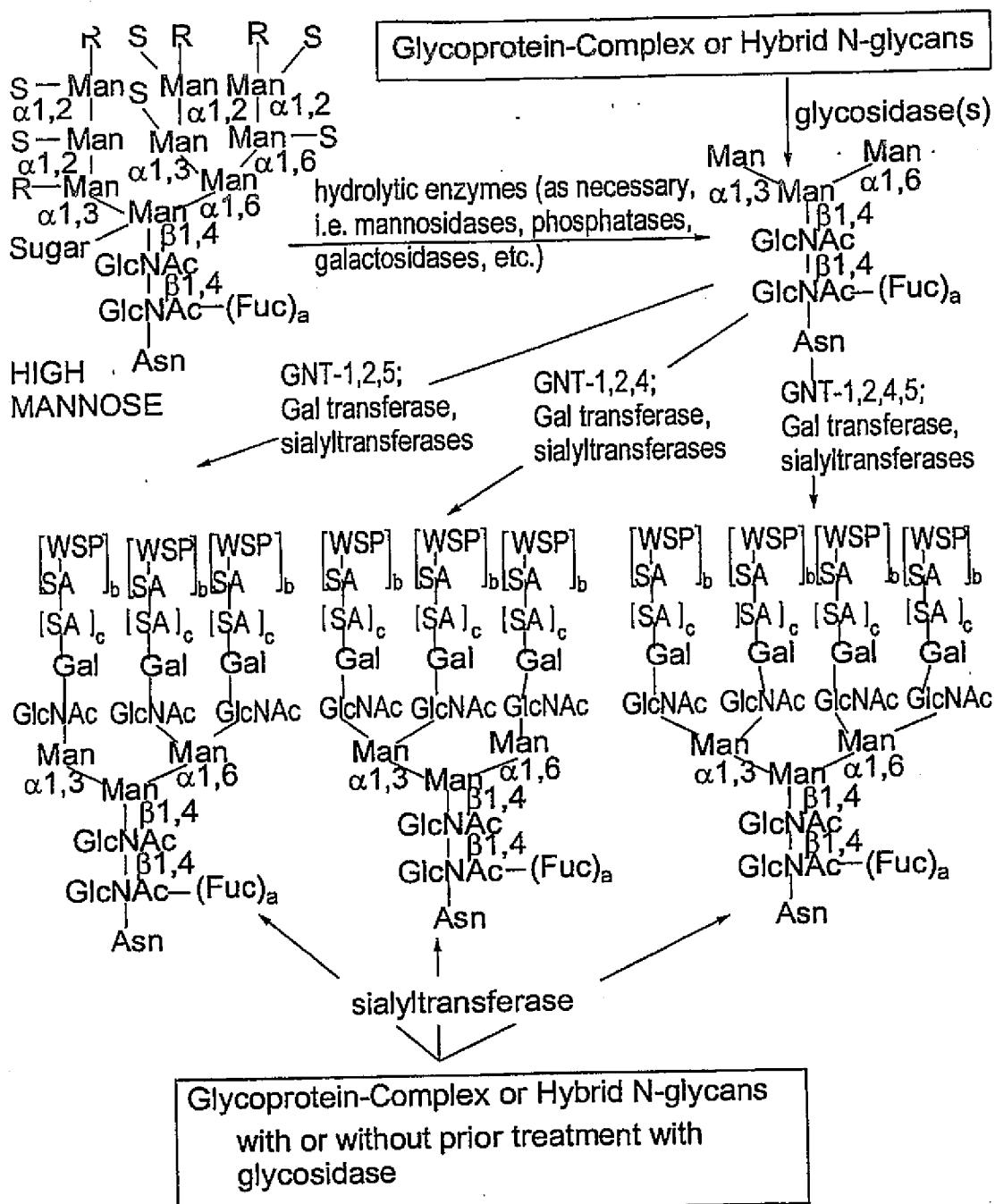
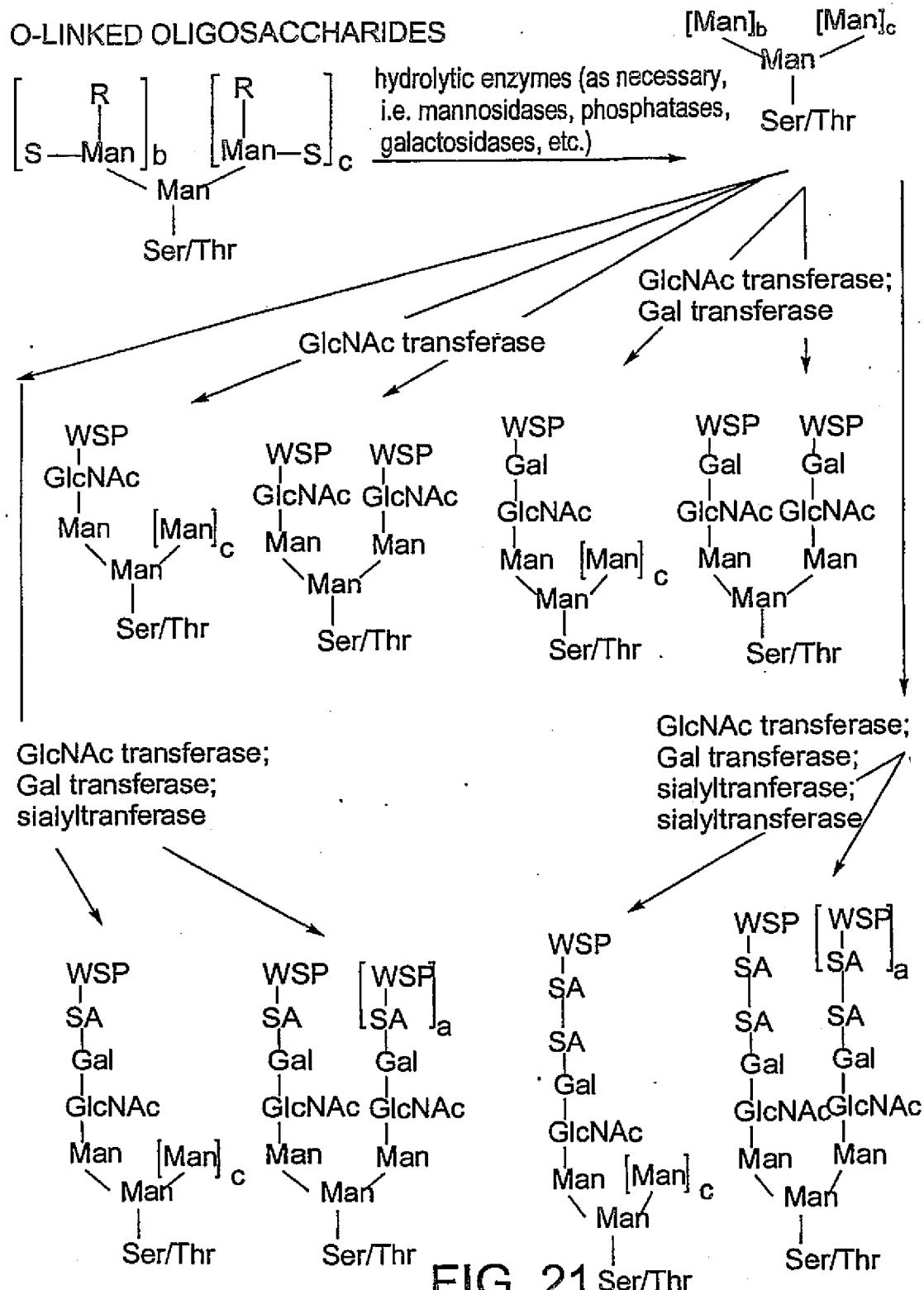


FIG. 20



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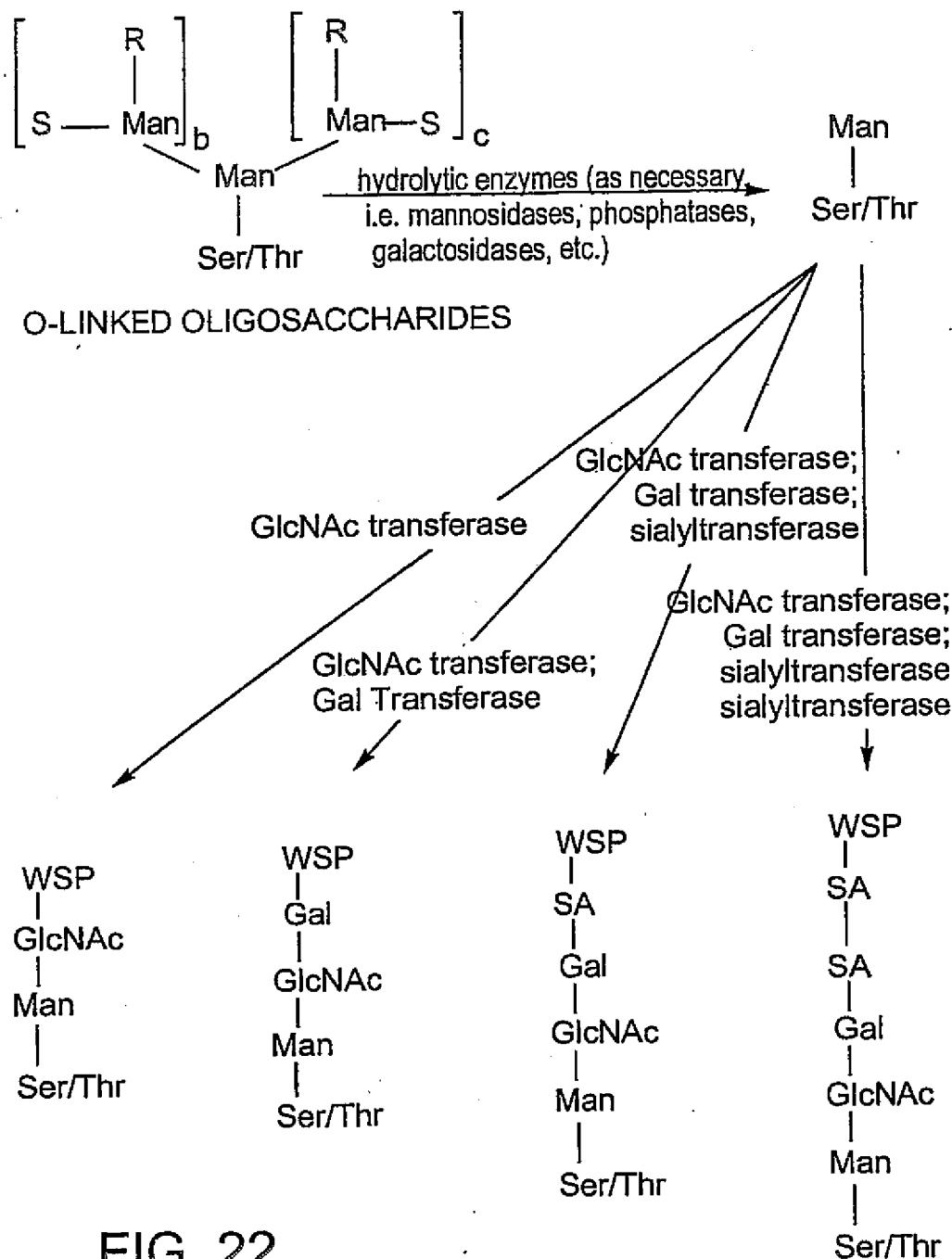


FIG. 22

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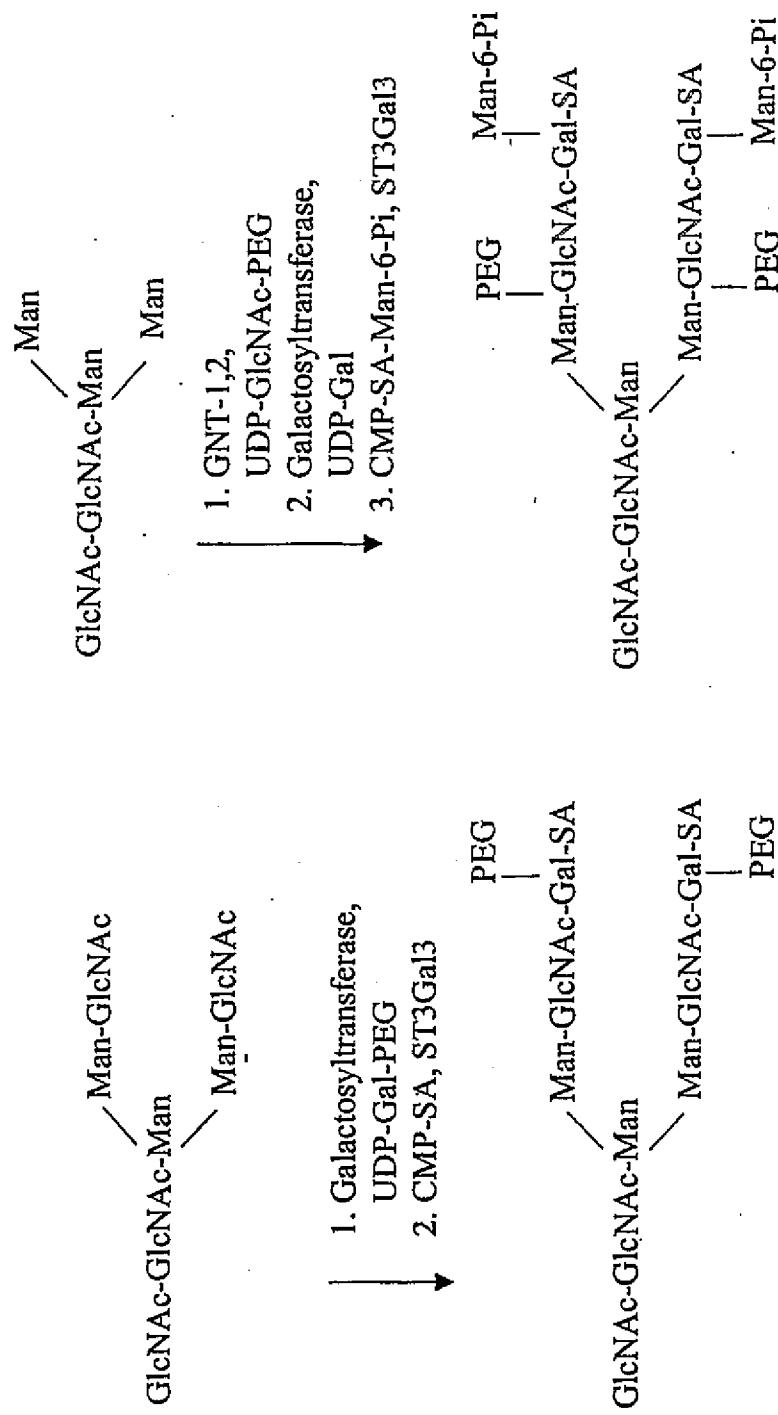


FIG. 23A  
FIG. 23B

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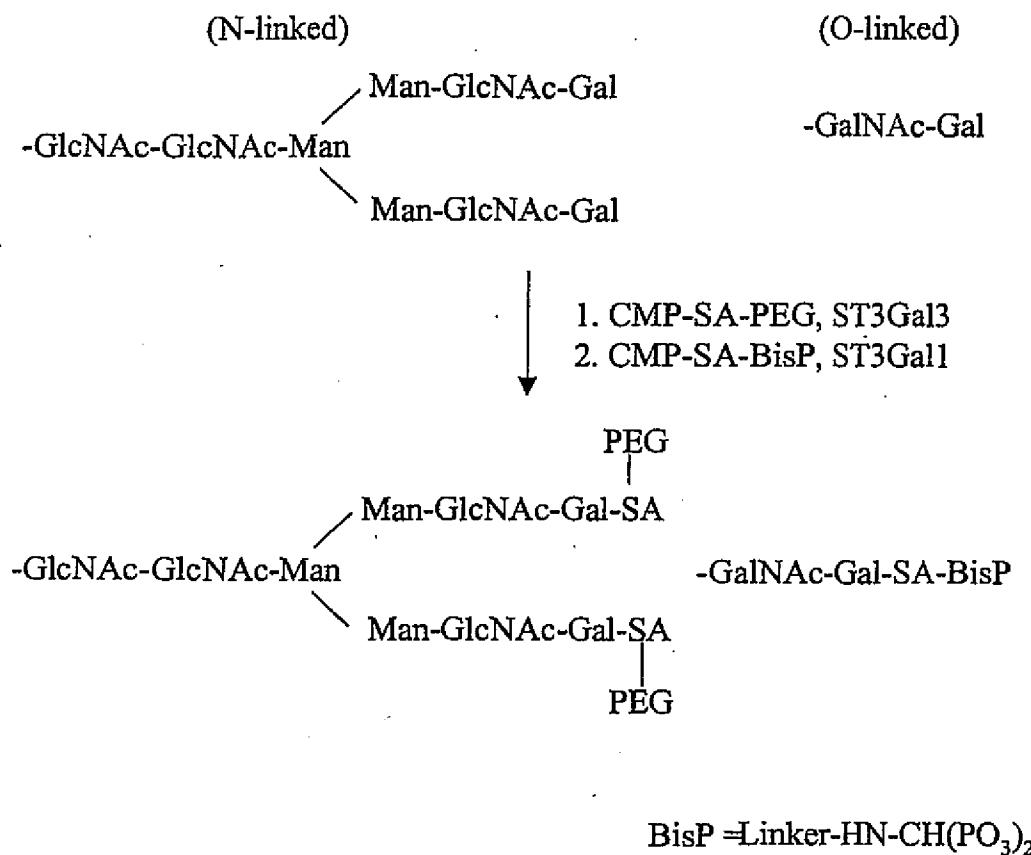


FIG. 23C

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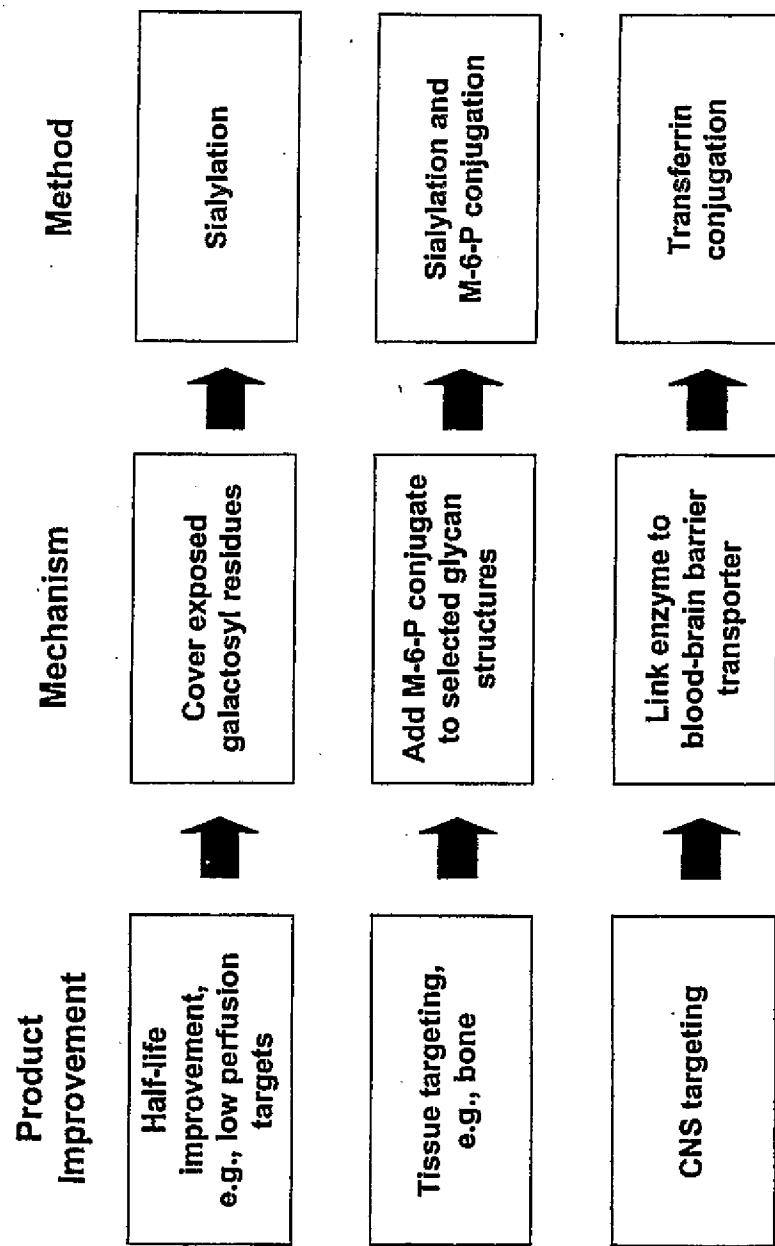


FIG. 24

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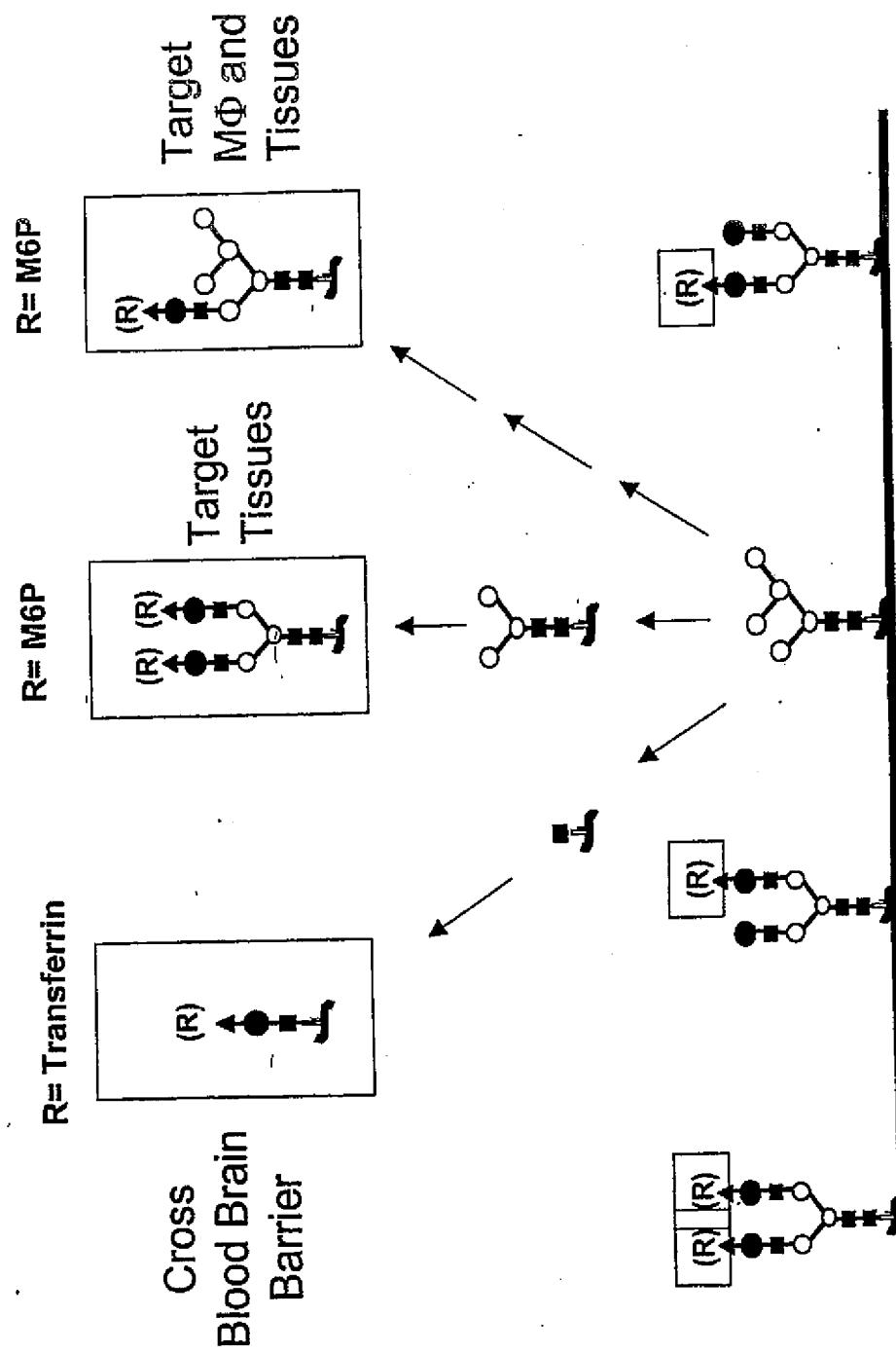


FIG. 25

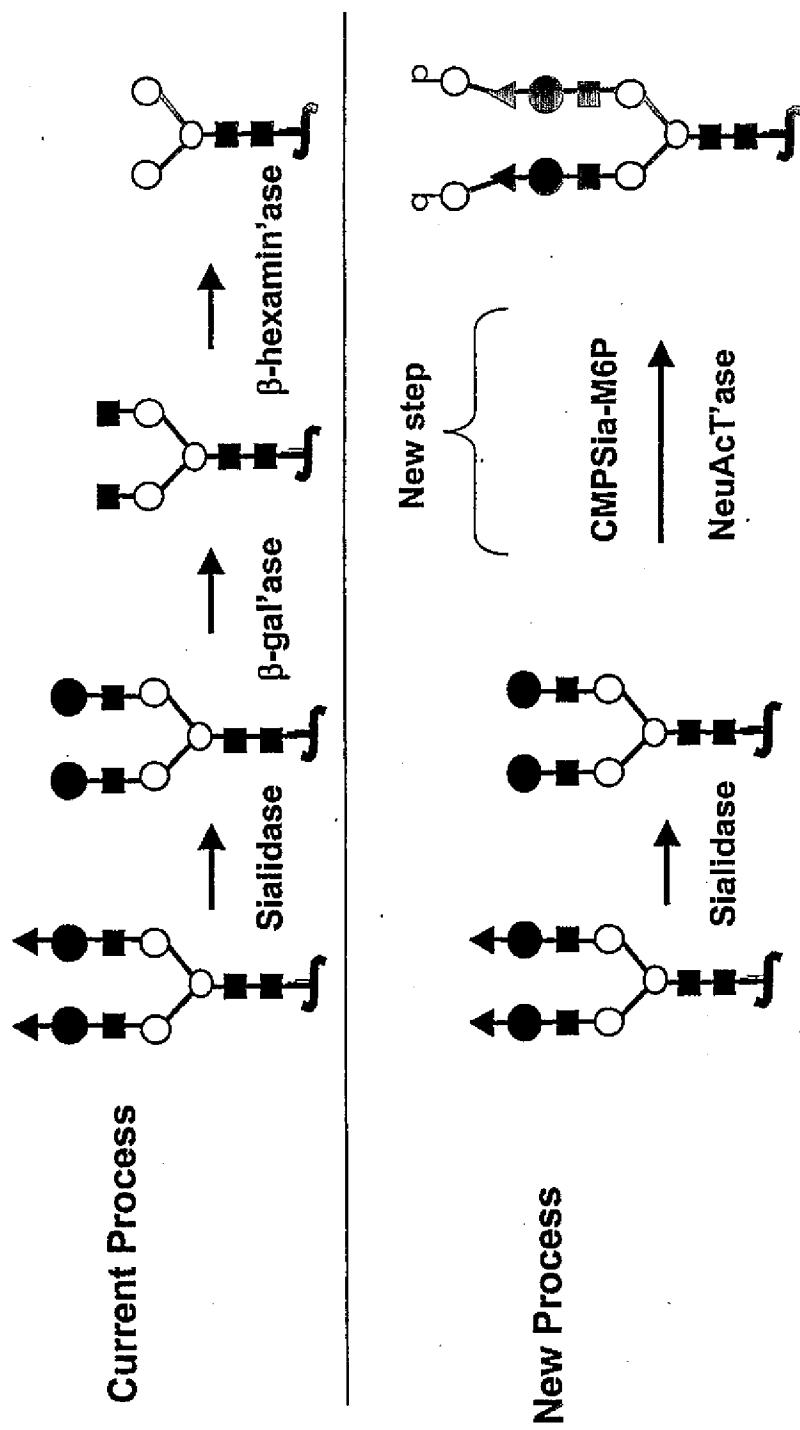


FIG. 26

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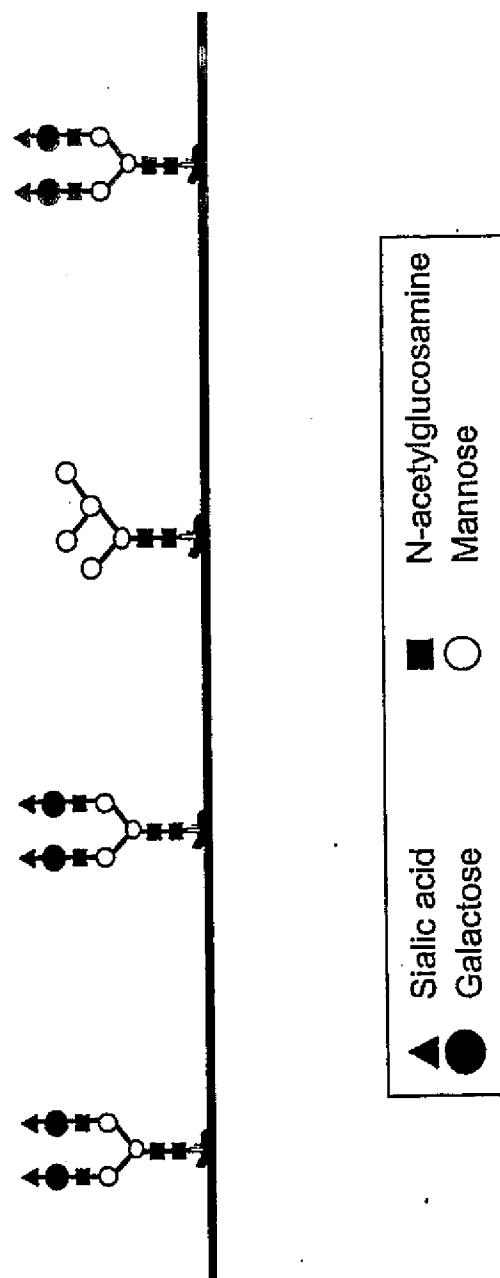


FIG. 27

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12AP1/E5 -- Viventia Biotech	AI-201 – AutoImmune
1964 -- Aventis	AI-301 – AutoImmune
20K growth hormone -- AMUR	AIDS vaccine – ANRS, CIBG, Hesed
28P6/E6 -- Viventia Biotech	Biomed, Hollis-Eden, Rome, United
3-Hydroxyphthaloyl-beta-lactoglobulin –	Biomedical, American Home Products,
4-IBB ligand gene therapy –	Maxygen
64-Cu MAb conjugate TETA-1A3 --	airway receptor ligand – IC Innovations
Mallinckrodt Institute of Radiology	AJvW 2 – Ajinomoto
64-Cu MAb conjugate TETA-cT84.66	AK 30 NGF -- Alkermes
64-Cu Trastuzumab TETA conjugate –	Albuferon -- Human Genome Sciences
Genentech	albumin – Biogen, DSM Anti-Infectives,
A 200 -- Amgen	Genzyme Transgenics, PPL Therapeutics,
A10255 – Eli Lilly	TranXenoGen, Welfide Corp.
A1PDX – Hederal Therapeutics	aldesleukin -- Chiron
A6 -- Angstrom	alefacept -- Biogen
aaAT-III -- Genzyme	Alemtuzumab
Abciximab – Centocor	Allergy therapy -- ALK-Abello/Maxygen,
ABI.001 – Atlantic BioPharmaceuticals	ALK-Abello/RP Scherer
ABT-828 – Abbott	allergy vaccines -- Allergy Therapeutics
Accutin	Alnidofibatide -- Aventis Pasteur
Actinohivin	Alnorine -- SRC VB VECTOR
activin – Biotech Australia, Human	ALP 242 -- Gruenthal
Therapeutics, Curis	Alpha antitrypsin -- Arriva/Hyland
AD 439 – Tanox	Immuno/ProMetic/Protease Sciences
AD 519 – Tanox	Alpha-1 antitrypsin – Cutter, Bayer, PPL
Adalimumab -- Cambridge Antibody Tech.	Therapeutics, Profile, ZymoGenetics,
Adenocarcinoma vaccine – Biomira -- NIS	Arriva
Adenosine deanimase -- Enzond	Alpha-1 protease inhibitor -- Genzyme
Adenosine A2B receptor antagonists --	Transgenics, Welfide Corp.
Adenosine Therapeutics	Alpha-galactose fusion protein –
ADP-001 – Axis Genetics	immunomedics
AF 13948 – Affymax	Alpha-galactosidase A -- Research
Afelimomab – Knoll	Corporation Technologies, Genzyme
AFP-SCAN – Immunomedics	Alpha-glucosidase – Genzyme, Novazyme
AG 2195 – Corixa	Alpha-lactalbumin
agalsidase alfa -- Transkaryotic Therapies	Alpha-L-iduronidase -- Transkaryotic
agalsidase beta -- Genzyme	Therapies, BioMarin
AGENT – Antisoma	alteplase – Genentech
AI 300 – AutoImmune	alvircept sudotox -- NIH
AI-101 – Teva	ALX1-11 – sNPS Pharmaceuticals
AI-102 – Teva	Alzheimer's disease gene therapy

FIG. 28A

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AM-133 -- AMRAD	Anti-angiogenesis monoclonal antibodies --
Amb a 1 immunostim conj. -- Dynavax	KS Biomedix/Schering AG
AMD 3100 -- AnorMED -- NIS	Anti-B4 MAb-DC1 conjugate -- ImmunoGen
AMD 3465 -- AnorMED -- NIS	Anti-B7 antibody PRIMATIZED -- IDEC
AMD 3465 -- AnorMED -- NIS	Anti-B7-1 MAb 16-10A1
AMD Fab -- Genentech	Anti-B7-1 MAb 1G10
Amediplase -- Menarini, Novartis	Anti-B7-2 MAb GL-1
AM-F9	Anti-B7-2-gelonin immunotoxin --
Amoebiasis vaccine	Antibacterials/antifungals --
Amphiregulin -- Octagene	Diversa/IntraBiotics
anakinra -- Amgen	Anti-beta-amyloid monoclonal antibodies --
analgesic -- Nobex	Cambridge Antibody Tech., Wyeth-Ayerst
ancestim -- Amgen	Anti-BLyS antibodies -- Cambridge
AnergiX.RA -- Corixa, Organon	Antibody Tech. /Human Genome Sciences
Angiocidin -- InKine	Antibody-drug conjugates -- Seattle
angiogenesis inhibitors -- ILEX	Genetics/Eos
AngioMab -- Antisoma	Anti-C5 MAb BB5-1 -- Alexion
Angiopoietins -- Regeneron/Procter & Gamble	Anti-C5 MAb N19-8 -- Alexion
angiostatin -- EntreMed	Anti-C8 MAb
Angiostatin/endostatin gene therapy -- Genetix Pharmaceuticals	anticancer cytokines -- BioPulse
angiotensin-II, topical -- Maret	anticancer matrix -- Telios Integra
Anthrax -- EluSys Therapeutics/US Army Medical Research Institute	Anticancer monoclonal antibodies -- ARIUS, Immunex
Anthrax vaccine	anticancer peptides -- Maxygen, Micrologix
Anti platelet-derived growth factor D human monoclonal antibodies -- CuraGen	Anticancer prodrug Tech. -- Alexion
Anti-17-1A MAb 3622W94 -- GlaxoSmithKline	Antibody Technologies
Anti-2C4 MAb -- Genentech	anticancer Troy-Bodies -- Affite -- Affitech
anti-4-1BB monoclonal antibodies -- Bristol- Myers Squibb	anticancer vaccine -- NIH
Anti-Adhesion Platform Tech. -- Cytovax	anticancers -- Epimmune
Anti-adipocyte MAb -- Cambridge Antibody Tech./ObeSys	Anti-CCR5/CXCR4 sheep MAb -- KS Biomedix Holdings
antiallergics -- Maxygen	Anti-CD11a MAb KBA --
antiallergy vaccine -- Acambis	Anti-CD11a MAb M17
Anti-alpha-4-integrin MAb	Anti-CD11a MAb TA-3 --
Anti-alphav $\beta$ 3 integrin MAb -- Applied Molecular Evolution	Anti-CD11a MAb WT.1 --
	Anti-CD11b MAb -- Pharmacia
	Anti-CD11b MAb LM2
	Anti-CD154 MAb -- Biogen
	Anti-CD16-anti-CD30 MAb -- Biotest
	Anti-CD18 MAb -- Pharmacia
	Anti-CD19 MAb B43 --

FIG. 28B

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Anti-CD19 MAb -liposomal sodium butyrate conjugate -	Anti-CD4 MAb 4162W94 – GlaxoSmithKline
Anti-CD147	Anti-CD4 MAb B-F5 – Diaclone
Anti-CD19 MAb-saporin conjugate –	Anti-CD4 MAb GK1-5
Anti-CD19-dsFv-PE38-immunotoxin –	Anti-CD4 MAb KT6
Anti-CD2 MAb 12-15 –	Anti-CD4 MAb OX38
Anti-CD2 MAb B-E2 – Diaclone	Anti-CD4 MAb PAP conjugate -- Bristol-Myers Squibb
Anti-CD2 MAb OX34 –	Anti-CD4 MAb RIB 5-2
Anti-CD2 MAb OX54 –	Anti-CD4 MAb W3/25
Anti-CD2 MAb OX55 –	Anti-CD4 MAb YTA 3.1.2
Anti-CD2 MAb RM2-1	Anti-CD4 MAb YTS 177-9
Anti-CD2 MAb RM2-2	Anti-CD40 ligand MAb 5c8 – Biogen
Anti-CD2 MAb RM2-4	Anti-CD40 MAb
Anti-CD20 MAb BCA B20	Anti-CD40 MAb 5D12 – Tanox
Anti-CD20-anti-Fc alpha RI bispecific MAb – Medarex, Tenovus	Anti-CD44 MAb A3D8
Anti-CD22 MAb-saporin-6 complex –	Anti-CD44 MAb GKWA3
Anti-CD3 immunotoxin –	Anti-CD44 MAb IM7
Anti-CD3 MAb 145-2C11 -- Pharming	Anti-CD44 MAb KM81
Anti-CD3 MAb CD4IgG conjugate -- Genentech	Anti-CD44 variant monoclonal antibodies -- Corixa/Hebrew University
Anti-CD3 MAb humanised – Protein Design, RW Johnson	Anti-CD45 MAb BC8-I-131
Anti-CD3 MAb WT32	Anti-CD45RB MAb
Anti-CD3 MAb-ricin-chain-A conjugate –	Anti-CD48 MAb HuLy-m3
Anti-CD3 MAb-xanthine-oxidase conjugate –	Anti-CD48 MAb WM-63
Anti-CD30 MAb BerH2 -- Medac	Anti-CD5 MAb -- Becton Dickinson
Anti-CD30 MAb-saporin conjugate	Anti-CD5 MAb OX19
Anti-CD30-scFv-ETA'-immunotoxin	Anti-CD6 MAb
Anti-CD38 MAb AT13/5	Anti-CD7 MAb-PAP conjugate
Anti-CD38 MAb-saporin conjugate	Anti-CD7 MAb-ricin-chain-A conjugate
Anti-CD3-anti-CD19 bispecific MAb	Anti-CD8 MAb – Amerimmune, Cytodyn, Becton Dickinson
Anti-CD3-anti-EGFR MAb	Anti-CD8 MAb 2-43
Anti-CD3-anti-interleukin-2-receptor MAb	Anti-CD8 MAb OX8
Anti-CD3-anti-MOV18 MAb – Centocor	Anti-CD80 MAb P16C10 -- IDEC
Anti-CD3-anti-SCLC bispecific MAb	Anti-CD80 MAb P7C10 -- ID Vaccine
Anti-CD4 idiotype vaccine	Anti-CD8-idarubicin conjugate
Anti-CD4 MAb – Centocor, IDEC Pharmaceuticals, Xenova Group	Anti-CEA MAb CE-25
Anti-CD4 MAb 16H5	Anti-CEA MAb MN 14 – Immunomedics
	Anti-CEA MAb MN14-PE40 conjugate – Immunomedics

FIG. 28C

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Anti-CEA MAb T84.66-interleukin-2 conjugate	Anti-heparanase human monoclonal antibodies -- Oxford Glycosciences/Medarex
Anti-CEA sheep MAb -- KS Biomedix Holdings	Anti-hepatitis C virus human monoclonal antibodies -- XTL Biopharmaceuticals
Anti-cell surface monoclonal antibodies -- Cambridge Antibody Tech. /Pharmacia	Anti-HER-2 antibody gene therapy
Anti-c-erbB2-anti-CD3 bifunctional MAb -- Otsuka	Anti-herpes antibody -- Epicyte
Anti-CMV MAb -- Scotgen	Anti-HIV antibody -- Epicyte
Anti-complement	anti-HIV catalytic antibody -- Hesed Biomed
Anti-CTLA-4 MAb	anti-HIV fusion protein -- Idun
Anti-EGFR catalytic antibody -- Hesed Biomed	anti-HIV proteins -- Cangene
anti-EGFR immunotoxin -- IVAX	Anti-HM1-24 MAb -- Chugai
Anti-EGFR MAb -- Abgenix	Anti-hR3 MAb
Anti-EGFR MAb 528	Anti-Human-Carcinoma-Antigen MAb -- Epicyte
Anti-EGFR MAb KSB 107 -- KS Biomedix	Anti-ICAM-1 MAb -- Boehringer Ingelheim
Anti-EGFR MAb-DM1 conjugate -- ImmunoGen	Anti-ICAM-1 MAb 1A-29 -- Pharmacia
Anti-EGFR MAb-LA1 --	Anti-ICAM-1 MAb HA58
Anti-EGFR sheep MAb -- KS Biomedix	Anti-ICAM-1 MAb YN1/1.7.4
Anti-FAP MAb F19-I-131	Anti-ICAM-3 MAb ICM3 -- ICOS
Anti-Fas IgM MAb CH11	Anti-idiotype breast cancer vaccine 11D10
Anti-Fas MAb Jo2	Anti-idiotype breast cancer vaccine
Anti-Fas MAb RK-8	ACA14C5 --
Anti-Flt-1 monoclonal antibodies -- ImClone	Anti-idiotype cancer vaccine -- ImClone
Anti-fungal peptides -- State University of New York	Systems/Merck KGaA ImClone, Viventia Biotech
antifungal tripeptides -- BTG	Anti-idiotype cancer vaccine 1A7 -- Titan
Anti-ganglioside GD2 antibody-interleukin-2 fusion protein -- Lexigen	Anti-idiotype cancer vaccine 3H1 -- Titan
Anti-GM2 MAb -- Kyowa	Anti-idiotype cancer vaccine TriAb -- Titan
Anti-GM-CSF receptor monoclonal antibodies -- AMRAD	Anti-idiotype Chlamydia trachomatis vaccine
Anti-gp130 MAb -- Tosoh	Anti-idiotype colorectal cancer vaccine -- Novartis
Anti-HCA monoclonal antibodies -- AltaRex/Epigen	Anti-idiotype colorectal cancer vaccine -- Onyxvax
Anti-hCG antibodies -- Abgenix/AVI BioPharma	Anti-idiotype melanoma vaccine -- IDEC Pharmaceuticals
	Anti-idiotype ovarian cancer vaccine ACA 125
	Anti-idiotype ovarian cancer vaccine AR54 - AltaRex

FIG. 28D

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Anti-idiotype ovarian cancer vaccine CA-125 – AltaRex, Biomira	Anti-L-selectin monoclonal antibodies -- Protein Design Labs, Abgenix, Stanford University
Anti-IgE catalytic antibody -- Hesed Biomed	Anti-MBL monoclonal antibodies -- Alexion/Brigham and Women's Hospital
Anti-IgE MAb E26 -- Genentech	Anti-MHC monoclonal antibodies
Anti-IGF-1 MAb	Anti-MIF antibody humanised -- IDEC, Cytokine PharmaSciences
anti-inflammatory -- GeneMax	Anti-MRSA/VRSA sheep MAb -- KS Biomedix Holdings
anti-inflammatory peptide -- BTG	Anti-mu MAb -- Novartis
anti-integrin peptides -- Burnham	Anti-MUC-1 MAb
Anti-interferon-alpha-receptor MAb 64G12 -- Pharma Pacific Management	Anti-MUC 18
Anti-interferon-gamma MAb -- Protein Design Labs	Anti-Nogo-A MAb IN1
Anti-interferon-gamma polyclonal antibody - - Advanced Biotherapy	Anti-nuclear autoantibodies -- Procyon
Anti-interleukin-10 MAb --	Anti-ovarian cancer monoclonal antibodies - - Dompe
Anti-interleukin-12 MAb --	Anti-p185 monoclonal antibodies
Anti-interleukin-1-beta polyclonal antibody -- R&D Systems	Anti-p43 MAb
Anti-interleukin-2 receptor MAb 2A3	Antiparasitic vaccines
Anti-interleukin-2 receptor MAb 33B3-1 -- Immunotech	Anti-PDGF/bFGF sheep MAb -- KS Biomedix
Anti-interleukin-2 receptor MAb ART-18	Anti-properdin monoclonal antibodies -- Abgenix/Gliatech
Anti-interleukin-2 receptor MAb LO-Tact-1	Anti-PSMA (prostrate specific membrane antigen)
Anti-interleukin-2 receptor MAb Mikbeta1	Anti-PSMA MAb J591 -- BZL Biologics
Anti-interleukin-2 receptor MAb NDS61	Anti-Rev MAb gene therapy --
Anti-interleukin-4 MAb 11B11	Anti-RSV antibodies -- Epicyte, Intracell
Anti-interleukin-5 MAb -- Wallace Laboratories	Anti-RSV monoclonal antibodies --
Anti-interleukin-6 MAb -- Centocor, Diacclone, Pharmadigm	Medarex/MedImmune, Applied Molecular Evolution/MedImmune
Anti-interleukin-8 MAb -- Abgenix	Anti-RSV MAb, inhalation --
Anti-interleukin-8 MAb -- Xenotech	Alkermes/MedImmune
Anti-JL1 MAb	Anti-RT gene therapy
Anti-Klebsiella sheep MAb -- KS Biomedix Holdings	Antisense K-ras RNA gene therapy
Anti-Laminin receptor MAb-liposomal doxorubicin conjugate	Anti-SF-25 MAb
Anti-LCG MAb -- Cytoclonal	Anti-sperm antibody -- Epicyte
Anti-lipopolysaccharide MAb -- VitaResc	Anti-Tac(Fv)-PE38 conjugate
	Anti-TAPA/CD81 MAb AMP1
	Anti-tat gene therapy

FIG. 28E

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Anti-TCR-alphabeta MAb H57-597	AOP-RANTES – Senetek
Anti-TCR-alphabeta MAb R73	Apan-CH – Praecis Pharmaceuticals
Anti-tenascin MAb BC-4-I-131	APC-8024 – Demegen
Anti-TGF-beta human monoclonal antibodies -- Cambridge Antibody Tech., Genzyme	ApoA-1 -- Milano, Pharmacia
Anti-TGF-beta MAb 2G7 – Genentech	Apogen -- Alexion
Antithrombin III – Genzyme Transgenics, Aventis, Bayer, Behringwerke, CSL, Myriad	apolipoprotein A1 – Avanir
Anti-Thy1 MAb	Apolipoprotein E – Bio-Tech. General
Anti-Thy1.1 MAb	Applaggin – Biogen
Anti-tissue factor/factor VIIa sheep MAb – KS Biomedix	aprotinin – ProdiGene
Anti-TNF monoclonal antibodies – Centocor, Chiron, Peptech, Pharacia, Serono	APT-070C – AdProTech
Anti-TNF sheep MAb -- KS Biomedix Holdings	AR 177 – Aronex Pharmaceuticals
Anti-TNFalpha MAb -- Genzyme	AR 209 – Aronex Pharmaceuticals, Antigenics
Anti-TNFalpha MAb B-C7 -- Diaclone	AR545C
Anti-tooth decay MAb -- Planet BioTech.	ARGENT gene delivery systems – ARIAD
Anti-TRAIL receptor-1 MAb -- Takeda	Arresten
Antitumour RNases – NIH	ART-123 – Asahi Kasei
Anti-VCAM MAb 2A2 -- Alexion	arylsulfatase B -- BioMarin
Anti-VCAM MAb 3F4 -- Alexion	Arylsulfatase B, Recombinant human -- BioMarin
Anti-VCAM-1 MAb	AS 1051 – Ajinomoto
Anti-VEC MAb – ImClone	ASI-BCL – Intracell
Anti-VEGF MAb -- Genentech	Asparaginase - Merck
Anti-VEGF MAb 2C3	ATL-101 – Alizyme
Anti-VEGF sheep MAb -- KS Biomedix Holdings	Atrial natriuretic peptide – Pharis
Anti-VLA-4 MAb HP1/2 -- Biogen	Aurintricarboxylic acid-high molecular weight
Anti-VLA-4 MAb PS/2	Autoimmune disorders -- GPC
Anti-VLA-4 MAb R1-2	Biotech/MorphoSys
Anti-VLA-4 MAb TA-2	Autoimmune disorders and transplant rejection -- Bristol-Myers Squibb/Genzyme Tra
Anti-VAP-1 human MAb	Autoimmune disorders/cancer -- Abgenix/Chiron, CuraGen
Anti-VRE sheep MAb -- KS Biomedix Holdings	Autotaxin
ANUP -- TranXenoGen	Avicidin – NeoRx
ANUP-1 – Pharis	axogenesis factor-1 -- Boston Life Sciences
	Axokine – Regeneron
	B cell lymphoma vaccine – Biomira
	B7-1 gene therapy –
	BABS proteins – Chiron

FIG. 28F

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BAM-002 -- Novelos Therapeutics	BMP 2 -- Genetics Institute/Medtronic-
Basiliximab (anti CD25 MAb) -- Novartis	Sofamor Danek, Genetics Institute/
Bay-16-9996 -- Bayer	Collagenesis, Genetics
Bay-39-9437 -- Bayer	Institute/Yamanouch
Bay-50-4798 -- Bayer	BMP 2 gene therapy
BB-10153 -- British Biotech	BMP 52 -- Aventis Pasteur, Biopharm
BBT-001 -- Bolder BioTech.	BMP-2 -- Genetics Institute
BBT-002 -- Bolder BioTech.	BMS 182248 -- Bristol-Myers Squibb
BBT-003 -- Bolder BioTech.	BMS 202448 -- Bristol-Myers Squibb
BBT-004 -- Bolder BioTech.	bone growth factors -- IsoTis
BBT-005 -- Bolder BioTech.	BPC-15 -- Pfizer
BBT-006 -- Bolder BioTech.	brain natriuretic peptide --
BBT-007 -- Bolder BioTech.	Breast cancer -- Oxford
BCH-2763 -- Shire	GlycoSciences/Medarex
BCSF -- Millenium Biologix	Breast cancer vaccine -- Therion Biologics,
BDNF -- Regeneron -- Amgen	Oregon
Becaplermin -- Johnson & Johnson, Chiron	BSSL -- PPL Therapeutics
Bectumomab -- Immunomedics	BST-2001 -- BioStratum
Beriplast -- Aventis	BST-3002 -- BioStratum
Beta-adrenergic receptor gene therapy -- University of Arkansas	BTI 322 --
bFGF -- Scios	butyrylcholinesterase -- Shire
BI 51013 -- Behringwerke AG	C 6822 -- COR Therapeutics
BIBH 1 -- Boehringer Ingelheim	C1 esterase inhibitor -- Pharming
BIM-23190 -- Beaufour-Ipsen	C3d adjuvant -- AdProTech
birch pollen immunotherapy -- Pharmacia	CAB-2.1 -- Millennium
bispecific fusion proteins -- NIH	calcitonin -- Inhale Therapeutics Systems,
Bispecific MAb 2B1 -- Chiron	Aventis, Genetronics, TranXenoGen,
Bitistatin	Unigene, Rhone Poulenc Rohrer
BIWA 4 -- Boehringer Ingelheim	calcitonin -- oral -- Nobex, Emisphere,
blood substitute -- Northfield, Baxter Intl.	Pharmaceutical Discovery
BLP-25 -- Biomira	Calcitonin gene-related peptide -- Asahi
BLS-0597 -- Boston Life Sciences	Kasei -- Unigene
BLyS -- Human Genome Sciences	calcitonin, human -- Suntory
BLyS radiolabelled -- Human Genome Sciences	calcitonin, nasal -- Novartis, Unigene
BM 06021 -- Boehringer Mannheim	calcitonin, Panoderm -- Elan
BM-202 -- BioMarin	calcitonin, Peptitrol -- Shire
BM-301 -- BioMarin	calcitonin, salmon -- Therapicon
BM-301 -- BioMarin	calin -- Biopharm
BM-302 -- BioMarin	Calphobindin I
	calphobindin I -- Kowa
	calreticulin -- NYU

FIG. 28G

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Campath-1G	CD4 fusion toxin -- Senetek
Campath-1M	CD4 IgG -- Genentech
cancer therapy -- Cangene	CD4 receptor antagonists -- Pharmacopeia/Progenics
cancer vaccine -- Aixlie, Aventis Pasteur, Center of Molecular Immunology ,YM BioSciences, Cytos, Genzyme, Transgenics, GlobeImmune, Igeneon, ImClone, Virogenetics, InterCell, Iomai, Jenner Biotherapies, Memorial Sloan-Kettering Cancer Center, Sydney Kimmel Cancer Center, Novavax, Protein Sciences, Argonex, SIGA	CD4 soluble -- Progenics
Cancer vaccine ALVAC-CEA B7.1 -- Aventis Pasteur/Therion Biologics	CD4, soluble -- Genzyme Transgenics
Cancer vaccine CEA-TRICOM -- Aventis Pasteur/Therion Biologics	CD40 ligand -- Immunex
Cancer vaccine gene therapy -- Cantab Pharmaceuticals	CD4-ricin chain A -- Genentech
Cancer vaccine HER-2/neu -- Corixa	CD59 gene therapy -- Alexion
Cancer vaccine THERATOPE -- Biomira	CD8 TIL cell therapy -- Aventis Pasteur
cancer vaccine, PolyMASC -- Valentis	CD8, soluble -- Avidex
Candida vaccine -- Corixa, Inhibitex	CD95 ligand -- Roche
Canstatin -- ILEX	CDP 571 -- Celltech
CAP-18 -- Panorama	CDP 850 -- Celltech
Cardiovascular gene therapy -- Collateral Therapeutics	CDP-860 (PEG-PDGF MAb) -- Celltech
carperotide -- Suntory	CDP 870 -- Celltech
Casocidin-1 -- Pharis	CDS-1 -- Ernest Orlando
CAT 152 -- Cambridge Antibody Tech.	Cedelizumab -- Ortho-McNeil
CAT 192 -- Cambridge Antibody Tech.	Cetermin -- Insmed
CAT 213 -- Cambridge Antibody Tech.	CETP vaccine -- Avant
Catalase-- Enzon	Cetorelix
Cat-PAD -- Circassia	Cetuximab
CB 0006 -- Celltech	CGH 400 -- Novartis
CCK(27-32)-- Akzo Nobel	CGP 42934 -- Novartis
CCR2-64I -- NIH	CGP 51901 -- Tanox
CD, Procept -- Paligent	CGRP -- Unigene
CD154 gene therapy	CGS 27913 -- Novartis
CD39 -- Immunex	CGS 32359 -- Novartis
CD39-L2 -- Hyseq	Chagas disease vaccine -- Corixa
CD39-L4 -- Hyseq	chemokines -- Immune Response
	CHH 380 -- Novartis
	chitinase -- Genzyme, ICOS
	Chlamydia pneumoniae vaccine -- Antex Biologics
	Chlamydia trachomatis vaccine -- Antex Biologics
	Chlamydia vaccine -- GlaxoSmithKline
	Cholera vaccine CVD 103-HgR -- Swiss Serum and Vaccine Institute Berne
	Cholera vaccine CVD 112 -- Swiss Serum and Vaccine Institute Berne

FIG. 28H

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Cholera vaccine inactivated oral – SBL Vaccin	CRL 1605 – CytRx CS-560 – Sankyo
Chrysalin -- Chrysalis BioTech.	CSF – ZymoGenetics
CI-782 – Hitachi Kase	CSF-G – Hangzhou, Dong-A, Hanmi
Ciliary neurotrophic factor -- Fidia, Roche	CSF-GM – Cangene, Hunan, LG Chem
CIM project – Active Biotech	CSF-M – Zarix
CL 329753 – Wyeth-Ayerst	CT 1579 – Merck Frosst
CL22, Cobra – ML Laboratories	CT 1786 – Merck Frosst
Clenoliximab – IDEC	CT-112 <sup>A</sup> – BTG
Clostridium difficile antibodies – Epicycle	CTB-134L – Xenova
clotting factors -- Octagene	CTC-111 – Kaketsuken
CMB 401 – Celltech	CTGF – FibroGen
CNTF – Sigma-Tau	CTLA4-Ig – Bristol-Myers Squibb
Cocaine abuse vaccine – Cantab, ImmuLogic, Scripps	CTLA4-Ig gene therapy – CTP-37 – AVI BioPharma
coccidiomycosis vaccine -- Arizo	C-type natriuretic peptide – Suntory
collagen – Type I – Pharming	CVS 995 – Corvas Intl.
Collagen formation inhibitors – FibroGen	CX 397 – Nikko Kyodo
Collagen/hydroxyapatite/bone growth factor – Aventis Pasteur, Biopharm, Orquest	CY 1747 – Epimmune CY 1748 -- Epimmune
collagenase -- BioSpecifics	Cyanovirin-N
Colorectal cancer vaccine -- Wistar Institute	Cystic fibrosis therapy -- CBR/IVAX
Component B, Recombinant -- Serono	CYT 351
Connective tissue growth factor inhibitors -- FibroGen/Taisho	cytokine Traps -- Regeneron cytokines – Enzon, Cytoclonal
Contortrostatin	Cytomegalovirus glycoprotein vaccine – Chiron, Aquila Biopharmaceuticals,
contraceptive vaccine -- Zonagen	Aventis Pasteur, Virogenetics
Contraceptive vaccine hCG	Cytomegalovirus vaccine live – Aventis Pasteur
Contraceptive vaccine male reversible -- IMMUCON	Cytosine deaminase gene therapy – GlaxoSmithKline
Contraceptive vaccine zona pellucida – Zonagen	DA-3003 – Dong-A
Copper-64 labelled MAb TETA-1A3 -- NCI	DAB389interleukin-6 – Senetek
Coralyne	DAB389interleukin-7
Corsevin M	Daclizumab (anti-IL2R MAb) – Protein Design Labs
C-peptide analogues -- Schwarz	DAMP <sup>A</sup> – Incyte Genomics
CPI-1500 – Consensus	Danplestim -- Pharmacia
CRF -- Neurobiological Tech.	darbepoetin alfa -- Amgen
cRGDFV pentapeptide –	DBI-3019 – Diabetogen
CRL 1095 – CytRx	
CRL 1336 – CytRx	

FIG. 28I

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DCC -- Genzyme	Duteplase -- Baxter Intl.
DDF -- Hyseq	DWP-401 -- Daewoong
decorin -- Integra, Telios	DWP-404 -- Daewoong
defensins -- Large Scale Biology	DWP-408 -- Daewoong
DEGR-VIIa	Dx 88 (Epi-KAL2) -- Dyax
Delmmunised antibody 3B6/22 AGEN	Dx 890 (elastin inhibitors) -- Dyax
Deimmunised anti-cancer antibodies -- Biovation/Viragen	E coli O157 vaccine -- NIH
Dendroamide A	E21-R -- BresaGen
Dengue vaccine -- Bavarian Nordic, Merck	Eastern equine encephalitis virus vaccine --
denileukin diftitox -- Ligand	Echicetin --
DES-1101 -- Desmos	Echinhibin 1 --
desirudin -- Novartis	Echistatin -- Merck
desmopressin -- Unigene	Echitamine --
Desmoteplase -- Merck, Schering AG	Ecromeximab -- Kyowa Hakko
Destabilase	EC-SOD -- PPL Therapeutics
Diabetes gene therapy -- DeveloGen, Pfizer	Eculizumab (5G1.1) -- Alexion
Diabetes therapy -- Crucell	EDF -- Ajinomoto
Diabetes type 1 vaccine -- Diamyd Therapeutics	EDN derivative -- NIH
DiaCIM -- YM BioSciences	EDNA -- NIH
dialytic oligopeptides -- Research Corp	Edobacombab -- XOMA
Diamyd -- Diamyd Therapeutics	Edrecolomab -- Centocor
DiaPep227-- Pepgen	EF 5077
DiavaX -- Corixa	Efalizumab -- Genentech
Digoxin MAb -- Glaxo	EGF fusion toxin -- Seragen, Ligand
Diphtheria tetanus pertussis-hepatitis B vaccine -- GlaxoSmithKline	EGF-P64k vaccine -- Center of Molecular Immunology
DIR therapy -- Solis Therapeutics --	EL 246 -- LigoCyte
DNase -- Genentech	elastase inhibitor -- Synergen
Dornase alfa -- Genentech	elcatonin -- Therapicon
Dornase alfa, inhalation -- Genentech	EMD 72000 -- Merck KGaA
Doxorubicin-anti-CEA MAb conjugate -- Immunomedics	Emdogain -- BIORA
DP-107 -- Trimeris	emfilermin -- AMRAD
drotrecogin alfa -- Eli Lilly	Emoctakin -- Novartis
DTctGMCSF	enamel matrix protein -- BIORA
DTP-polio vaccine -- Aventis Pasteur	Endo III -- NYU
DU 257-KM231 antibody conjugate -- Kyowa	endostatin -- EntreMed, Pharis
dural graft matrix -- Integra	Enhancins -- Micrologix
	Enlimomab -- Isis Pharm.
	Enoxaparin sodium -- Pharmuka
	enzyme linked antibody nutrient depletion therapy -- KS Biomedix Holdings

FIG. 28J

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Eosinophil-derived neutralizing agent -- EP-51216 -- Asta Medica	Factor VII -- Novo Nordisk, Bayer, Baxter Intl.
EP-51389 -- Asta Medica	Factor VIIa -- PPL Therapeutics, ZymoGenetics
EPH family ligands -- Regeneron	Factor VIII -- Bayer Genentech, Beaufour Ipsen, CLB, Inex, Octagen, Pharmacia, Pharming
Epidermal growth factor -- Hitachi Kasei, Johnson & Johnson	Factor VIII -- PEGylated -- Bayer
Epidermal growth factor fusion toxin -- Senetek	Factor VIII fragments -- Pharmacia
Epidermal growth factor-genistein -- EPI-HNE-4 -- Dyax	Factor VIII gene therapy -- Targeted Genetics
EPI-KAL2 -- Dyax	Factor VIII sucrose formulation -- Bayer, Genentech
Epoetin-alfa -- Amgen, Dragon Pharmaceuticals, Nanjing Huaxin	Factor VIII-2 -- Bayer
Epratuzumab -- Immunomedics	Factor VIII-3 -- Bayer
Epstein-Barr virus vaccine -- Aviron/SmithKline Beecham, Bioresearch	Factor Xa inhibitors -- Merck, Novo Nordisk, Mochida
Eptacog alfa -- Novo Nordisk	Factor XIII -- ZymoGenetics
Eptifibatide -- COR Therapeutics	Factors VIII and IX gene therapy -- Genetics Institute/Targeted Genetics
erb-38 --	erythropoietin -- Alkermes, ProLease, Dong-Famoxin -- Genset
Erlizumab -- Genentech	Fas (delta) TM protein -- LXR BioTech.
erythropoietin -- Alkermes, ProLease, Dong-Famoxin -- Genset	Fas TR -- Human Genome Sciences
A, Elanex, Genetics Institute, LG Chem, Protein Sciences, Serono, Snow Brand, SRC VB VECTOR, Transkaryotic Therapies	Felvizumab -- Scotgen
Erythropoietin Beta -- Hoffman La Roche	FFR-VIIa -- Novo Nordisk
Erythropoietin/Epoetin alfa -- Chugai	FG-001 -- F-Gene
Escherichia coli vaccine -- North American Vaccine, SBL Vaccin, Swiss Serum and Vaccine Institute Berne	FG-002 -- F-Gene
etanercept -- Immunex	FG-004 -- F-Gene
examorelin -- Mediolanum	FG-005 -- F-Gene
Exendin 4 -- Amylin	FGF + fibrin -- Repair
exonuclease VII	Fibrimage -- Bio-Tech. General
F 105 -- Centocor	fibrin-binding peptides -- ISIS Innovation
F-992 -- Fornix	fibrinogen -- PPL Therapeutics, Pharming
Factor IX -- Alpha Therapeutics, Welfide Corp., CSL, Genetics Institute/AHP, Pharmacia, PPL Therapeutics	fibroblast growth factor -- Chiron, NYU, Ramot, ZymoGenetics
Factor IX gene therapy -- Cell Genesys	fibrolase conjugate -- Schering AG
	Filgrastim -- Amgen
	filgrastim -- PDA modified -- Xencor
	FLT-3 ligand -- Immunex
	FN18 CRM9 --

FIG. 28K

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follistatin -- Biotech Australia, Human Therapeutics	glutamate decarboxylase -- Genzyme Transgenics
follitropin alfa -- Alkermes, ProLease, PowderJect, Serono, Akzo Nobel	Glycoprotein S3 -- Kureha
Follitropin Beta -- Bayer, Organon	GM-CSF -- Immunex
FP 59	GM-CSF tumour vaccine -- PowderJect
FSH -- Ferring	GnRH immunotherapeutic -- Protherics
FSH + LH -- Ferring	Goserelin (LhRH antagonist) -- AstraZeneca
F-spondin -- CeNeS	gp75 antigen -- ImClone
fusion protein delivery system -- UAB Research Foundation	gp96 -- Antigenics
fusion toxins -- Boston Life Sciences	GP10100 -- Galenica
G 5598 -- Genentech	GR 4991W93 -- GlaxoSmithKline
GA-II -- Transkaryotic Therapies	Granulocyte colony-stimulating factor -- Dong-A
Gamma-interferon analogues -- SRC VB VECTOR	Granulocyte colony-stimulating factor conjugate
Ganirelix -- Roche	grass allergy therapy -- Dynavax
gastric lipase -- Meristem	GRF1-44 -- ICN
Gavilimomab --	Growth Factor -- Chiron, Atrigel, Atrix, Innogenetics, ZymoGenetics, Novo
G-CSF -- Amgen, SRC VB VECTOR	growth factor peptides -- Biotherapeutics
GDF-1 -- CeNeS	growth hormone -- LG Chem
GDF-5 -- Biopharm	growth hormone, Recombinant human -- Serono
GDNF (glial derived neurotrophic factor) -- Amgen	GT 4086 -- Giatech
gelsolin -- Biogen	GW 353430 -- GlaxoSmithKline
Gemtuzumab ozogamicin -- Celltech	GW-278884 -- GlaxoSmithKline
Gene-activated epoetin-alfa -- Aventis Pharma -- Transkaryotic Therapies	H 11 -- Viventia Biotech
Glanzmann thrombasthenia gene therapy --	H5N1 influenza A virus vaccine -- Protein Sciences
Glatiramer acetate -- Yeda	haemoglobin -- Biopure
glial growth factor 2 -- CeNeS	haemoglobin 3011, Recombinant -- Baxter Healthcare
GLP-1 -- Amylin, Suntory, TheraTech, Watson	haemoglobin crofumaryl -- Baxter Intl.
GLP-1 peptide analogues -- Zealand Pharmaceuticals	haemoglobin stabilized -- Ajinomoto
glucagon -- Eli Lilly, ZymoGenetics	haemoglobin, recombinant -- Apex
Glucagon-like peptide-1 7-36 amide -- Suntory	HAF -- Immune Response
Glucogen-like peptide -- Amylin	Hantavirus vaccine
Glucocerebrosidase -- Genzyme	HB 19
	HBNF -- Regeneron
	HCC-1 -- Pharis
	hCG -- Milkhaus

FIG. 28L

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hCG vaccine – Zonagen	Herpes simplex glycoprotein DNA vaccine –
HE-317 – Hollis-Eden Pharmaceuticals	Merck, Wyeth-Lederle Vaccines-Malvern,
Heat shock protein cancer and influenza	Genentech, GlaxoSmithKline, Chiron,
vaccines -- StressGen	Takeda
Helicobacter pylori vaccine -- Acambis,	Herpes simplex vaccine -- Cantab
AstraZeneca/CSL, Chiron, Provalis	Pharmaceuticals, CEL-SCI, Henderson
Helistat-G – GalaGen	Morley
Hemolink – Hemosol	Herpes simplex vaccine live -- ImClone
hepapoietin -- Snow Brand	Systems/Wyeth-Lederle, Aventis Pasteur
heparanase – InSight	HGF derivatives -- Dompe
heparinase I -- Ibex	hIAPP vaccine -- Crucell
heparinase III – Ibex	Hib-hepatitis B vaccine -- Aventis Pasteur
Hepatitis A vaccine -- American Biogenetic	HIC 1
Sciences	HIP – Altachem
Hepatitis A vaccine inactivated	Hirudins – Biopharma, Cangene, Dongkook,
Hepatitis A vaccine Nothav – Chiron	Japan Energy Corporation, Pharmacia
Hepatitis A-hepatitis B vaccine –	Corporation, SIR International, Sanofi-
GlaxoSmithKline	Synthelabo, Sotragene, Rhein Biotech
hepatitis B therapy -- Tripep	HIV edible vaccine -- ProdiGene
Hepatitis B vaccine – Amgen, Chiron SpA,	HIV gp120 vaccine – Chiron, Ajinomoto,
Meiji Milk, NIS, Prodeva, PowderJect,	GlaxoSmithKline, ID Vaccine, Progenics,
Rhein Biotech	VaxGen
Hepatitis B vaccine recombinant -- Evans	HIV gp120 vaccine gene therapy –
Vaccines, Epitec Combiotech, Genentech,	HIV gp160 DNA vaccine – PowderJect,
MedImmune, Merck Sharp & Dohme,	Aventis Pasteur, Oncogen, Hyland
Rhein Biotech, Shantha Biotechnics,	Immuno, Protein Sciences
Vector, Yeda	HIV gp41 vaccine -- Panacos
Hepatitis B vaccine recombinant TGP 943	HIV HGP-30W vaccine – CEL-SCI
- Takeda	HIV immune globulin – Abbott, Chiron
Hepatitis C vaccine – Bavarian Nordic,	HIV peptides – American Home Products
Chiron, Innogenetics Acambis,	HIV vaccine – Applied bioTech., Axis
Hepatitis D vaccine -- Chiron Vaccines	Genetics, Biogen, Bristol-Myers Squibb,
Hepatitis E vaccine recombinant –	Genentech, Korea Green Cross, NIS,
Genelabs/GlaxoSmithKline, Novavax	Oncogen, Protein Sciences Corporation,
hepatocyte growth factor – Panorama,	Terumo, Tonen Corporation, Wyeth-
Sosei	Ayerst, Wyeth-Lederle Vaccines-Malvern,
hepatocyte growth factor kringle fragments -	Advanced BioScience Laboratories,
- EntreMed	Bavarian Nordic, Bavarian Nordic/Statens
Her-2/Neu peptides – Corixa	Serum Institute, GeneCure, Immune
	Response, Progenics, Therion Biologics,
	United Biomedical, Chiron

FIG. 28M

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HIV vaccine vCP1433 -- Aventis Pasteur	Human monoclonal antibodies --
HIV vaccine vCP1452 -- Aventis Pasteur	Medarex/Northwest Biotherapeutics,
HIV vaccine vCP205 -- Aventis Pasteur	Medarex/Seattle Genetics
HL-9 -- American BioScience	human netrin-1 -- Exelixis
HM-9239 -- Cytran	human papillomavirus antibodies -- Epicycle
HML-103 -- Hemosol	Human papillomavirus vaccine -- Biotech
HML-104 -- Hemosol	Australia, IDEC, StressGen
HML-105 -- Hemosol	Human papillomavirus vaccine MEDI 501 --
HML-109 -- Hemosol	MedImmune/GlaxoSmithKline
HML-110 -- Hemosol	Human papillomavirus vaccine MEDI
HML-121 -- Hemosol	503/MEDI 504 --
hNLP -- Pharis	MedImmune/GlaxoSmithKline
Hookworm vaccine	Human papillomavirus vaccine TA-CIN --
host-vector vaccines -- Henogen	Cantab Pharmaceuticals
HPM 1 -- Chugai	Human papillomavirus vaccine TA-HPV --
HPV vaccine -- MediGene	Cantab Pharmaceuticals
HSA -- Meristem	Human papillomavirus vaccine TH-GW --
HSF -- StressGen	Cantab/GlaxoSmithKline
HSP carriers --Weizmann, Yeda, Peptor	human polyclonal antibodies -- Biosite/Eos
HSPPC-70 -- Antigenics	BioTech./ Medarex
HSPPC-96, pathogen-derived -- Antigenics	human type II anti factor VIII monoclonal
HSV 863 -- Novartis	antibodies -- ThromboGenics
HTLV-I DNA vaccine	humanised anti glycoprotein Ib murine
HTLV-I vaccine	monoclonal antibodies -- ThromboGenics
HTLV-II vaccine -- Access	HumaRAD -- Intracell
HU 901 -- Tanox	HuMax EGFR -- Genmab
Hu23F2G -- ICOS	HuMax-CD4 -- Medarex
HuHMFG1	HuMax-IL15 -- Genmab
HumaLYM -- Intracell	HYB 190 -- Hybridon
Human krebs statika -- Yamanouchi	HYB 676 -- Hybridon
human monoclonal antibodies --	I-125 MAb A33 -- Celltech
Abgenix/Biogen, Abgenix/ Corixa,	Ibritumomab tiuxetan -- IDEC
Abgenix/Immunex, Abgenix/Lexicon,	IBT-9401 -- Ibex
Abgenix/ Pfizer, Athersys/Medarex,	IBT-9402 -- Ibex
Biogen/MorphoSys, CAT/Searle,	IC 14 -- ICOS
Centocor/Medarex, Corixa/Kirin Brewery,	Idarubicin anti-Ly-2.1 --
Corixa/Medarex, Eos BioTech./Medarex,	IDE 114 -- IDEC
Eos/Xenerex, Exelixis/Protein Design	IDE 131 -- IDEC
Labs, ImmunoGen/ Raven, Medarex/	IDE 152 -- IDEC
B.Twelve, MorphoSys/ImmunoGen, XTL	IDM 1 -- IDM
Biopharmaceuticals/Dyax,	IDPS -- Hollis-Eden Pharmaceuticals

FIG. 28N

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iduronate-2-sulfatase -- Transkaryotic Therapies	insulin -- Autoimmune, Altea, Biobras, BioSante, Bio-Tech. General, Chong Kun Dang, Emissphere, Flamel, Provalis, Rhein Biotech, TranXenoGen
IGF/IBP-2-13 -- Pharis	insulin (bovine) -- Novartis
IGN-101 -- Igeneon	insulin analogue -- Eli Lilly
IK HIR02 -- Iketon	Insulin Aspart -- Novo Nordisk
IL-11 -- Genetics Institute/AHP	insulin detemir -- Novo Nordisk
IL-13-PE38 -- NeoPharm	insulin glargine -- Aventis
IL-17 receptor -- Immunex	insulin inhaled -- Inhale Therapeutics
IL-18BP -- Yeda	Systems, Alkermes
IL-1Hy1 -- Hyseq	insulin oral -- Inovax
IL-1 $\beta$ -- Celltech	insulin, AeroDose -- AeroGen
IL-1 $\beta$ adjuvant -- Celltech	insulin, AERx -- Aradigm
IL-2 -- Chiron	insulin, BEODAS -- Elan
IL-2 + IL-12 -- Hoffman La-Roche	insulin, Biphasix -- Helix
IL-6/sIL-6R fusion -- Hadasit	insulin, buccal -- Generex
IL-6R derivative -- Tosoh	insulin, I2R -- Flemington
IL-7-Dap 389 fusion toxin -- Ligand	insulin, intranasal -- Bentley
IM-862 -- Cytran	insulin, oral -- Nobex, Unigene
IMC-1C11 -- ImClone	insulin, Orasome -- Endorex
imiglucerase -- Genzyme	insulin, ProMaxx -- Epic
immune globulin intravenous (human) -- Hoffman La Roche	insulin, Quadrant -- Elan
immune privilege factor -- Proneuron	insulin, recombinant -- Aventis
Immunocal -- Immunotec	insulin, Spiros -- Elan
Immunogene therapy -- Briana Bio-Tech	insulin, Transfersome -- IDEA
Immunoliposomal 5-fluorodeoxyuridine-dipalmitate --	insulin, Zymo, recombinant -- Novo Nordisk
immunosuppressant vaccine -- Aixlie	insulinotropin -- Scios
immunotoxin -- Antisoma, NIH	Insulysin gene therapy --
ImmuRAIT-Re-188 -- Immunomedics	integrin antagonists -- Merck
imreg-1 -- Imreg	interferon (Alpha2) -- SRC VB VECTOR, Viragen, Dong-A, Hoffman La-Roche, Genentech
infertility -- Johnson & Johnson, E-TRANS	interferon -- BioMedicines, Human Genome Sciences
Infliximab -- Centocor	interferon (Alfa-n3) -- Interferon Sciences Intl.
Influenza virus vaccine -- Aventis Pasteur, Protein Sciences	interferon (Alpha), Biphasix -- Helix
inhibin -- Biotech Australia, Human Therapeutics	
Inhibitory G protein gene therapy	
INKP-2001 -- InKine	
Inolimomab -- Diaclone	

FIG. 28O

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interferon (Alpha)—Amgen, BioNative, Novartis, Genzyme Transgenics, Hayashibara, Inhale Therapeutics Systems, Medusa, Flamel, Dong-A, GeneTrol, Nastech, Shantha, Wassermann, LG Chem, Sumitomo, Aventis, Behring EGIS, Pepgen, Servier, Rhein Biotech,  
 interferon (Alpha2A)  
 interferon (Alpha2B) — Enzon, Schering-Plough, Biogen, IDEA  
 interferon (Alpha-N1) — GlaxoSmithKline  
 interferon (beta) — Rentschler, GeneTrol, Meristem, Rhein Biotech, Toray, Yeda, Daiichi, Mochida  
 interferon (Beta1A) — Serono, Biogen  
 interferon (beta1A),inhale -- Biogen  
 interferon ( $\beta$ 1b)-- Chiron  
 interferon ( $\tau$ u)— Pepgen  
 Interferon alfacon-1 — Amgen  
 Interferon alpha-2a vaccine  
 Interferon Beta 1b — Schering/Chiron, InterMune  
 Interferon Gamma — Boehringer Ingelheim, Sheffield, Rentschler, Hayashibara  
 interferon receptor , Type I — Serono  
 interferon(Gamma1B) — Genentech  
 Interferon-alpha-2b + ribavirin — Biogen, ICN  
 Interferon-alpha-2b gene therapy -- Schering-Plough  
 Interferon-con1 gene therapy --  
 interleukin-1 antagonists — Dompe  
 Interleukin-1 receptor antagonist — Abbott Bioresearch, Pharmacia  
 Interleukin-1 receptor type I — Immunex  
 interleukin-1 receptor Type II -- Immunex  
 Interleukin-1 trap — Regeneron  
 Interleukin-1-alpha — Immunex/Roche  
 interleukin-2 — SRC VB VECTOR, Ajinomoto, Biomira, Chiron  
 IL-2/ diphtheria toxin — Ligand  
 Interleukin-3 — Cangene  
 Interleukin-4 — Immunology Ventures, Sanofi Winthrop, Schering-Plough, Immunex/ Sanofi Winthrop, Bayer, Ono  
 interleukin-4 + TNF-Alpha — NIH  
 interleukin-4 agonist — Bayer  
 interleukin-4 fusion toxin — Ligand  
 Interleukin-4 receptor — Immunex, Immun  
 Interleukin-6 — Ajinomoto, Cangene, Yeda, Genetics Institute, Novartis  
 interleukin-6 fusion protein  
 Interleukin-6 fusion toxin — Ligand, Serono  
 interleukin-7 -- IC Innovations  
 interleukin-7 receptor -- Immunex  
 interleukin-8 antagonists -- Kyowa Hakko/Millennium/Pfizer  
 interleukin-9 antagonists -- Genaera  
 Interleukin-10 — DNAX, Schering-Plough  
 Interleukin-10 gene therapy --  
 interleukin-12 -- Genetics Institute, Hoffman La-Roche  
 interleukin-13 -- Sanofi  
 interleukin-13 antagonists -- AMRAD  
 Interleukin-13-PE38QQR  
 interleukin-15 — Immunex  
 interleukin-16 — Research Corp  
 interleukin-18 — GlaxoSmithKline  
 Interleukin-18 binding protein -- Serono  
 Ior-P3 -- Center of Molecular Immunology  
 IP-10 — NIH  
 IPF -- Metabolex  
 IR-501 -- Immune Response  
 ISIS 9125 -- Isis Pharmaceuticals  
 ISURF No. 1554 -- Millennium  
 ISURF No. 1866 -- Iowa State Univer.  
 ITF-1697 -- Italfarmaco  
 IxC 162 -- Ixion  
 J 695 -- Cambridge Antibody Tech., Genetics Inst., Knoll  
 Jagged + FGF -- Repair

FIG. 28P

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JKC-362 – Phoenix Pharmaceuticals	leptin, 2nd-generation – Amgen
JTP-2942 – Japan Tobacce	leridistim – Pharmacia
Juman monoclonal antibodies --	leuprolide, ProMaxx -- Epic
Medarex/Raven	leuprorelin, oral -- Unigene
K02 – Axys Pharmaceuticals	LeuTech – Papatin
Keliximab – IDEC	LEX 032 – SuperGen
Keyhole limpet haemocyanin	LiDEPT – Novartis
KGF – Amgen	Lintuzumab (anti-CD33 MAb) -- Protein Design Labs
KM 871 – Kyowa	lipase – Altus Biologics
KPI 135 -- Scios	lipid A vaccine -- EntreMed
KPI-022 -- Scios	lipid-linked anchor Tech. – ICRT, ID Biomedical
Kringle 5	liposome-CD4 Tech. – Sheffield
KSB 304	Listeria monocytogenes vaccine
KSB-201 -- KS Biomedix	LMB 1
L 696418 -- Merck	LMB 7
L 703801 -- Merck	LMB 9 -- Battelle Memorial Institute, NIH
L1 – Acorda	LM-CD45 -- Cantab Pharmaceuticals
L-761191 -- Merck	lovastatin -- Merck
lactoferrin – Meristem, Pharming, Agennix	LSA-3
lactoferrin cardio – Pharming	LT- $\beta$ receptor -- Biogen
LAG-3 -- Serono	lung cancer vaccine – Corixa
LAIT – GEMMA	lusupultide -- Scios
LAK cell cytotoxin -- Arizona	L-Vax -- AVAX
lamellarins – PharmaMar/University of Malaga	LY 355455 – Eli Lilly
laminin A peptides -- NIH	LY 366405 – Eli Lilly
lanoteplase -- Genetics Institute	LY-355101 -- Eli Lilly
laronidase -- BioMarin	Lyme disease DNA vaccine – Vical/Aventis Pasteur
Lassa fever vaccine	Lyme disease vaccine -- Aquila
LCAT -- NIH	Biopharmaceuticals, Aventis, Pasteur, Symbicomb, GlaxoSmithKline, Hyland Immuno, MedImmune
LDP 01 -- Millennium	Lymphocytic choriomeningitis virus vaccine
LDP 02 -- Millennium	lymphoma vaccine – Biomira, Genitope
Lecithinized superoxide dismutase -- Seikagaku	LYP18
LeIF adjuvant – Corixa	lys plasminogen, recombinant
leishmaniasis vaccine -- Corixa	Lysosomal storage disease gene therapy -- Avigen
lenercept – Hoffman La-Roche	lysostaphin -- Nutrition 21
Lenograstim -- Aventis, Chugai	
lepirudin -- Aventis	
leptin – Amgen, IC Innovations	
Leptin gene therapy -- Chiron Corporation	

FIG. 28Q

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M 23 -- Gruenenthal	MEDI 507 -- BioTransplant
M1 monoclonal antibodies -- Acorda	melanin concentrating hormone --
Therapeutics	Neurocrine Biosciences
MA 16N7C2 -- Corvas Intl.	melanocortins -- OMRF
malaria vaccine -- GlaxoSmithKline,	Melanoma monoclonal antibodies -- Viragen
AdProTech, Antigenics, Apovia, Aventis	melanoma vaccine -- GlaxoSmithKline,
Pasteur, Axis Genetics, Behringwerke,	Akzo Nobel, Avant, Aventis Pasteur,
CDCP, Chiron Vaccines, Genzyme	Bavarian Nordic, Biovector, CancerVax,
Transgenics, Hawaii, MedImmune, NIH,	Genzyme Molecular Oncology, Humbolt,
NYU, Oxxon, Roche/Saramane, Biotech	ImClone Systems, Memorial, NYU, Oxxon
Australia, Rx Tech	Melanoma vaccine Magevac -- Therion
Malaria vaccine CDC/NIIMALVAC-1	memory enhancers -- Scios
malaria vaccine,multicomponent	meningococcal B vaccine -- Chiron
mammaglobin -- Corixa	meningococcal vaccine -- CAMR
mammastatin -- Biotherapeutics	Meningococcal vaccine group B conjugate -
mannan-binding lectin -- NatImm	- North American Vaccine
mannan-MUC1 -- Psiron	Meningococcal vaccine group B
MAP 30	recombinant -- BioChem Vaccines,
Marinovir -- Phytera	Microscience
MARstem -- Maret	Meningococcal vaccine group Y conjugate -
MB-015 -- Mochida	- North American Vaccine
MBP -- ImmuLogic	Meningococcal vaccine groups A B and C
MCI-028 -- Mitsubishi-Tokyo	conjugate -- North American Vaccine
MCIF -- Human Genome Sciences	Mepolizumab -- GlaxoSmithKline
MDC -- Advanced BioScience -- Akzo	Metastatin -- EntreMed, Takeda
Nobel, ICOS	Met-CkB7 -- Human Genome Sciences
MDX 11 -- Medarex	met-enkephalin -- TNI
MDX 210 -- Medarex	METH-1 -- Human Genome Sciences
MDX 22 -- Medarex	methioninase -- AntiCancer
MDX 22	Methionine lyase gene therapy --
MDX 240 -- Medarex	AntiCancer
MDX 33	Met-RANTES -- Genexa Biomedical,
MDX 44 -- Medarex	Serono
MDX 447 -- Medarex	Metreleptin
MDX H210 -- Medarex	Microtubule inhibitor MAb
MDX RA -- Houston BioTech., Medarex	Immunogen/Abgenix
ME-104 -- Pharmexa	MGDF -- Kirin
Measles vaccine	MGV -- Progenics
Mecasermin -- Cephalon/Chiron, Chiron	micrin -- Endocrine
MEDI 488 -- MedImmune	microplasmin -- ThromboGenics
MEDI 500	MIF -- Genetics Institute

FIG. 28R

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migration inhibitory factor -- NIH	MAb 45-2D9 -- haematoporphyrin conjugate
Mim CD4.1 -- Xycte Therapies	MAb 4B4
mirostipen -- Human Genome Sciences	MAb 4E3-CPA conjugate -- BCM Oncologia
Mitumomab (BEC-2) -- ImClone Systems, Merck KGaA	MAb 4E3-daunorubicin conjugate
MK 852 -- Merck	MAb 50-6
MLN 1202 (Anti-CCR2 monoclonal antibody) -- Millenium Pharmaceuticals	MAb 50-61A -- Institut Pasteur
Mobenakin -- NIS	MAb 5A8 -- Biogen
molgramostim -- Genetics Institute, Novartis	MAb 791T/36-methotrexate conjugate
monoclonal antibodies -- Abgenix/Celltech, Immusol/ Medarex, Viragen/ Roslin Institute, Cambridge Antibody Tech./Elan	MAb 7c11.e8
MAb 108 --	MAb 7E11 C5-selenocystamine conjugate
MAb 10D5 --	MAb 93KA9 -- Novartis
MAb 14.18-interleukin-2 immunocytokine -- Lexigen	MAb A5B7-cisplatin conjugate -- Biodynamics Research, Pharmacia
MAb 14G2a --	MAb A5B7-I-131
MAb 15A10 --	MAb A7
MAb 170 -- Biomira	MAb A717 -- Exocell
MAb 177Lu CC49 --	MAb A7-zinostatin conjugate
MAb 17F9	MAb ABX-RB2 -- Abgenix
MAb 1D7	MAb ACA 11
MAb 1F7 -- Immune Network	MAb AFP-I-131 -- Immunomedics
MAb 1H10-doxorubicin conjugate	MAb AP1
MAb 26-2F	MAb AZ1
MAb 2A11	MAb B3-LysPE40 conjugate
MAb 2E1 -- RW Johnson	MAb B4 -- United Biomedical
MAb 2F5	MAb B43 Genistein-conjugate
MAb 31.1 -- International Biolimmune Systems	MAb B43.13-Tc-99m -- Biomira
MAb 32 -- Cambridge Antibody Tech., Peptech	MAb B43-PAP conjugate
MAb 323A3 -- Centocor	MAb B4G7-gelonin conjugate
MAb 3C5	MAb BCM 43-daunorubicin conjugate -- BCM Oncologia
MAb 3F12	MAb BIS-1
MAb 3F8	MAb BMS 181170 -- Bristol-Myers Squibb
MAb 42/6	MAb BR55-2
MAb 425 -- Merck KGaA	MAb BW494
MAb 447-52D -- Merck Sharp & Dohme	MAb C 242-DM1 conjugate -- ImmunoGen
	MAb C242-PE conjugate
	MAb c30-6
	MAb CA208-cytorhodin-S conjugate -- Hoechst Japan
	MAb CC49 -- Enzon

FIG. 28S

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MAb ch14.18 --	MAb LL2-I-131 -- Immunomedics
MAb CH14.18-GM-CSF fusion protein --	MAb LL2-Y-90
Lexigen	MAb LS2D617 -- Hybritech
MAb chCE7	MAb LYM-1-gelonin conjugate
MAb CI-137 -- AMRAD	MAb LYM-1-I-131
MAb cisplatin conjugate	MAb LYM-1-Y-90
MAb CLB-CD19	MAb LYM-2 -- Peregrine
MAb CLB-CD19v	MAb M195
MAb CLL-1 -- Peregrine	MAb M195-bismuth 213 conjugate --
MAb CLL-1-GM-CSF conjugate	Protein Design Labs
MAb CLL-1-IL-2 conjugate -- Peregrine	MAb M195-gelonin conjugate
MAb CLN IgG -- doxorubicin conjugates	MAb M195-I-131
MAb conjugates -- Tanox	MAb M195-Y-90
MAb D612	MAb MA 33H1 -- Sanofi
MAb Dal B02	MAb MAD11
MAb DC101 -- ImClone	MAb MGb2
MAb EA 1 --	MAb MINT5
MAb EC708 -- Biovation	MAb MK2-23
MAb EP-5C7 -- Protein Design Labs	MAb MOC31 ETA(252-613) conjugate
MAb ERIC-1 -- ICRT	MAb MOC-31-In-111
MAb F105 gene therapy	MAb MOC-31-PE conjugate
MAb FC 2.15	MAb MR6 --
MAb G250 -- Centocor	MAb MRK-16 -- Aventis Pasteur
MAb GA6	MAb MS11G6
MAb GA733	MAb MX-DTPA BrE-3
MAb Gliomab-H -- Viventia Biotech	MAb MY9
MAb HB2-saporin conjugate	MAb Nd2 -- Tosoh
MAb HD 37 --	MAb NG-1 -- Hygeia
MAb HD37-ricin chain-A conjugate	MAb NM01 -- Nissin Food
MAb HNK20 -- Acambis	MAb OC 125
MAb huN901-DM1 conjugate --	MAb OC 125-CMA conjugate
ImmunoGen	MAb OKI-1 -- Ortho-McNeil
MAb I-131 CC49 -- Corixa	MAb OX52 -- Bioproducts for Science
MAb ICO25	MAb PMA5
MAb ICR12-CPG2 conjugate	MAb PR1
MAb ICR-62	MAb prost 30
MAb IRac-ricin A conjugate	MAb R-24
MAb K1	MAb R-24 a Human GD3 -- Celltech
MAb KS1-4-methotrexate conjugate	MAb RFB4-ricin chain A conjugate
MAb L6 -- Bristol-Myers Squibb, Oncogen	MAb RFT5-ricin chain A conjugate
MAb LiCO 16-88	MAb SC 1

FIG. 28T

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MAb SM-3 -- ICRT	Muc-1 vaccine -- Corixa
MAb SMART 1D10 -- Protein Design Labs	mucosal tolerance -- Aberdeen
MAb SMART ABL 364 -- Novartis	mullerian inhibiting subst
MAb SN6f	muplestim -- Genetics Institute, Novartis,
MAb SN6f-deglycosylated ricin A chain conjugate --	DSM Anti-Infectives
MAb SN6j	murine MAb -- KS Biomedix
MAb SN7-ricin chain A conjugate	Mutant somatropin -- JCR Pharmaceutical
MAb T101-Y-90 conjugate -- Hybritech	MV 833 -- Toagosei
MAb T-88 -- Chiron	Mycoplasma pulmonis vaccine
MAb TB94 -- Cancer ImmunoBiology	Mycoprex -- XOMA
MAb TEC 11	myeloperoxidase -- Henogen
MAb TES-23 -- Chugai	myostatin -- Genetics Institute
MAb TM31 -- Avant	Nacolomab tafenatox -- Pharmacia
MAb TNT-1 -- Cambridge Antibody Tech., Peregrine	Nagrestipen -- British Biotech
MAb TNT-3	NAP-5 -- Corvas Intl.
MAb TNT-3 -- IL2 fusion protein --	NAPc2 -- Corvas Intl.
MAb TP3-At-211	nartograstim -- Kyowa
MAb TP3-PAP conjugate --	Natalizumab -- Protein Design Labs
MAb UJ13A -- ICRT	Nateplase -- NIH, Nihon Schering
MAb UN3	nateplase -- Schering AG
MAb ZME-018-gelonin conjugate	NBI-3001 -- Neurocrine Biosci.
MAb-BC2 -- GlaxoSmithKline	NBI-5788 -- Neurocrine Biosci.
MAb-DM1 conjugate -- ImmunoGen	NBI-6024 -- Neurocrine Biosci.
MAb-ricin-chain-A conjugate -- XOMA	Nef inhibitors -- BRI
MAb-temoporfin conjugates	Neisseria gonorrhoea vaccine -- Antex Biologics
Monopharm C -- Viventia Biotech	Neomycin B-arginine conjugate
monteplase -- Eisai	Nerelimomab -- Chiron
montirelin hydrate -- Gruenthal	Nerve growth factor -- Amgen -- Chiron, Genentech
moroctocog alfa -- Genetics Institute	Nerve growth factor gene therapy
Moroctocog-alfa -- Pharmacia	nesiritide citrate -- Scios
MP 4	neuregulin-2 -- CeNeS
MP-121 -- Biopharm	neurocan -- NYU
MP-52 -- Biopharm	neuronal delivery system -- CAMR
MRA -- Chugai	Neurophil inhibitory Factor -- Corvas
MS 28168 -- Mitsui Chemicals, Nihon Schering	Neuroprotective vaccine -- University of Auckland
MSH fusion toxin -- Ligand	neurotrophic chimaeras -- Regeneron
MSI-99 -- Genaera	neurotrophic factor -- NsGene, CereMedix
MT 201 -- Micromet	

FIG. 28U

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NeuroVax -- Immune Response  
 neurutrin -- Genentech  
 neutral endopeptidase -- Genentech  
 NGF enhancers -- NeuroSearch  
 NHL vaccine -- Large Scale Biology  
 NIP45 -- Boston Life Sciences  
 NKI-B20  
 NM 01 -- Nissin Food  
 NMI-139 -- NitroMed  
 NMMP -- Genetics Institute  
 NN-2211 -- Novo Nordisk  
 Noggin -- Regeneron  
 Nonacog alfa  
 Norelin -- Biostar  
 Norwalk virus vaccine  
 NRLU 10 -- NeoRx  
 NRLU 10 PE -- NeoRx  
 NT-3 -- Regeneron  
 NT-4/5 -- Genentech  
 NU 3056  
 NU 3076  
 NX 1838 -- Gilead Sciences  
 NY ESO-1/CAG-3 antigen -- NIH  
 NYVAC-7 -- Aventis Pasteur  
 NZ-1002 -- Novazyme  
 obesity therapy -- Nobex  
 OC 10426 -- Ontogen  
 OC 144093 -- Ontogen  
 OCIF -- Sankyo  
 Oct-43 -- Otsuka  
 Odulimomab -- Immunotech  
 OK PSA - liposomal  
 OKT3-gamma-1-ala-ala  
 OM 991  
 OM 992  
 Omalizumab -- Genentech  
 oncoimmunin-L -- NIH  
 Oncolysin B -- ImmunoGen  
 Oncolysin CD6 -- ImmunoGen  
 Oncolysin M -- ImmunoGen  
 Oncolysin S -- ImmunoGen  
 Oncophage -- Antigenics  
 Oncostatin M -- Bristol-Myers Squibb  
 OncoVax-CL -- Jenner Biotherapies  
 OncoVax-P -- Jenner Biotherapies  
 onercept -- Yeda  
 onychomycosis vaccine -- Boehringer Ingelheim  
 opebecan -- XOMA  
 opioid -- Arizona  
 Oprelvekin -- Genetics Institute  
 Oregonomab -- AltaRex  
 Org-33408 b -- Akzo Nobel  
 Orolip DP -- EpiCept  
 oryzacystatin  
 OSA peptides -- GenSci Regeneration  
 osteoblast-cadherin GF -- Pharis  
 Osteocalcin-thymidine kinase gene therapy  
 osteogenic protein -- Curis  
 osteopontin -- OraPharma  
 osteoporosis peptides -- Integra, Telios  
 osteoprotegerin -- Amgen, SnowBrand  
 otitis media vaccines -- Antex Biologics  
 ovarian cancer -- University of Alabama  
 OX40-IgG fusion protein -- Cantab, Xenova  
 P 246 -- Diatide  
 P 30 -- Alfacell  
 p1025 -- Active Biotech  
 P-113<sup>A</sup> -- Demegen  
 P-16 peptide -- Transition Therapeutics  
 p43 -- Ramot  
 P-50 peptide -- Transition Therapeutics  
 p53 + RAS vaccine -- NIH, NCI  
 PACAP(1-27) analogue  
 paediatric vaccines -- Chiron  
 Pafase -- ICOS  
 PAGE-4 plasmid DNA -- IDEC  
 PAI-2 -- Biotech Australia, Human Therapeutics  
 Palifermin (keratinocyte growth factor) -- Amgen  
 Palivizumab -- MedImmune

FIG. 28V

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PAM 4 -- Merck	PEG-uricase -- Mountain View
pamiteplase -- Yamanouchi	Pegvisomant -- Genentech
pancreatin, Minitabs -- Eurand	PEGylated proteins, PolyMASC -- Valentis
Pangen -- Fournier	PEGylated recombinant native human leptin -- Roche
Pantarín -- Selective Genetics	Pemtumomab
Parainfluenza virus vaccine -- Pharmacia, Pierre Fabre	Penetratin -- Cyclacel
paraoxanase -- Esperion	Pepscan -- Antisoma
parathyroid hormone -- Abiogen, Korea Green Cross	peptide G -- Peptech, ICRT
Parathyroid hormone (1-34) -- Chugai/Suntory	peptide vaccine -- NIH ,NCI
Parkinson's disease gene therapy -- Cell Genesys/ Ceregene	Pexelizumab
Parvovirus vaccine -- MedImmune	pexiganan acetate -- Genaera
PCP-Scan -- Immunomedics	Pharmaprojects No. 3179 -- NYU
PDGF -- Chiron	Pharmaprojects No. 3390 -- Ernest Orlando
PDGF cocktail -- Theratechnologies	Pharmaprojects No. 3417 -- Sumitomo
peanut allergy therapy -- Dynavax	Pharmaprojects No. 3777 -- Acambis
PEG anti-ICAM MAb -- Boehringer Ingelheim	Pharmaprojects No. 4209 -- XOMA
PEG asparaginase -- Enzon	Pharmaprojects No. 4349 -- Baxter Intl.
PEG glucocerebrosidase	Pharmaprojects No. 4651
PEG hirudin -- Knoll	Pharmaprojects No. 4915 -- Avanir
PEG interferon-alpha-2a -- Roche	Pharmaprojects No. 5156 -- Rhizogenics
PEG interferon-alpha-2b + ribavirin -- Biogen, Enzon, ICN Pharmaceuticals, Schering-Plough	Pharmaprojects No. 5200 -- Pfizer
PEG MAb A5B7 -- Pegacaristim -- Amgen -- Kirin Brewery -- ZymoGenetics	Pharmaprojects No. 5215 -- Origene
Pegaldesleukin -- Research Corp	Pharmaprojects No. 5216 -- Origene
pegaspargase -- Enzon	Pharmaprojects No. 5218 -- Origene
pegfilgrastim -- Amgen	Pharmaprojects No. 5267 -- ML Laboratories
PEG-interferon Alpha -- Viragen	Pharmaprojects No. 5373 -- MorphoSys
PEG-interferon Alpha 2A -- Hoffman La-Roche	Pharmaprojects No. 5493 -- Metabolex
PEG-interferon Alpha 2B -- Schering-Plough	Pharmaprojects No. 5707 -- Genentech
PEG-r-hirudin -- Abbott	Pharmaprojects No. 5728 -- Autogen
PEG-rHuMGDF -- Amgen	Pharmaprojects No. 5733 -- BioMarin
	Pharmaprojects No. 5757 -- NIH
	Pharmaprojects No. 5765 -- Gryphon
	Pharmaprojects No. 5830 -- AntiCancer
	Pharmaprojects No. 5839 -- Dyax
	Pharmaprojects No. 5849 -- Johnson & Johnson
	Pharmaprojects No. 5860 -- Mitsubishi-Tokyo

FIG. 28W

Pharmaprojects No. 5869 -- Oxford GlycoSciences	Plasminogen activators -- Abbott Laboratories, American Home Products, Boehringer Mannheim, Chiron Corporation, DuPont Pharmaceuticals, Eli Lilly, Shionogi, Genentech, Genetics Institute, GlaxoSmithKline, Hemispherx Biopharma, Merck & Co, Novartis, Pharmacia Corporation, Wakamoto, Yeda
Pharmaprojects No. 5883 -- Asahi Brewery	plasminogen-related peptides -- Bio-Tech.
Pharmaprojects No. 5947 -- StressGen	General/MGH
Pharmaprojects No. 5961 -- Theratechnologies	platelet factor 4 -- RepliGen
Pharmaprojects No. 5962 -- NIH	Platelet-derived growth factor -- Amgen --
Pharmaprojects No. 5966 -- NIH	ZymoGenetics
Pharmaprojects No. 5994 -- Pharming	plusonermin -- Hayashibara
Pharmaprojects No. 5995 -- Pharming	PMD-2850 -- Protherics
Pharmaprojects No. 6023 -- IMMUCON	Pneumococcal vaccine -- Antex Biologics, Aventis Pasteur
Pharmaprojects No. 6063 -- Cytoclonal	Pneumococcal vaccine intranasal -- BioChem Vaccines/Biovector
Pharmaprojects No. 6073 -- SIDDCO	PR1A3
Pharmaprojects No. 6115 -- Genzyme	PR-39
Pharmaprojects No. 6227 -- NIH	pralmorelin -- Kaken
Pharmaprojects No. 6230 -- NIH	Pretarget-Lymphoma -- NeoRx
Pharmaprojects No. 6236 -- NIH	Priliximab -- Centocor
Pharmaprojects No. 6243 -- NIH	PRO 140 -- Progenics
Pharmaprojects No. 6244 -- NIH	PRO 2000 -- Procept
Pharmaprojects No. 6281 -- Senetek	PRO 367 -- Progenics
Pharmaprojects No. 6365 -- NIH	PRO 542 -- Progenics
Pharmaprojects No. 6368 -- NIH	pro-Apo A-I -- Esperion
Pharmaprojects No. 6373 -- NIH	prolactin -- Genzyme
Pharmaprojects No. 6408 -- Pan Pacific	Prosaptide TX14(A) -- Bio-Tech. General
Pharmaprojects No. 6410 -- Athersys	prostate cancer antibodies -- Immunex, UroCor
Pharmaprojects No. 6421 -- Oxford GlycoSciences	prostate cancer antibody therapy -- Genentech/UroGenesys, Genotherapeutics
Pharmaprojects No. 6522 -- Maxygen	prostate cancer immunotherapeutics -- The PSMA Development Company
Pharmaprojects No. 6523 -- Pharis	prostate cancer vaccine -- Aventis Pasteur, Zonagen, Corixa, Dendreon, Jenner Biotherapies, Therion Biologics
Pharmaprojects No. 6538 -- Maxygen	
Pharmaprojects No. 6554 -- APALEXO	
Pharmaprojects No. 6560 -- Ardana	
Pharmaprojects No. 6562 -- Bayer	
Pharmaprojects No. 6569 -- Eos	
Phenoxyazine	
Phenylase -- Ibex	
Pigment epithelium derived factor -- plasminogen activator inhibitor-1, recombinant -- DuPont Pharmaceuticals	

prostate-specific antigen -- EntreMed	RD 62198
protein A -- RepliGen	rDnase -- Genentech
protein adhesives -- Enzon	RDP-58 -- SangStat
protein C -- Baxter Intl., PPL Therapeutics, ZymoGenetics	RecepTox-Fce -- Keryx
protein C activator -- Gilead Sciences	RecepTox-GnRH -- Keryx, MTR
protein kinase R antags -- NIH	Technologies
protirelin -- Takeda	RecepTox-MBP -- Keryx, MTR
protocadherin 2 -- Caprion	Technologies
Pro-urokinase -- Abbott, Bristol-Myers Squibb, Dainippon, Tosoh -- Welfide	recFSH -- Akzo Nobel, Organon
P-selectin glycoprotein ligand-1 -- Genetics Institute	REGA 3G12
pseudomonal infections -- InterMune	Regavirumab -- Teijin
Pseudomonas vaccine -- Cytovax	relaxin -- Connetics Corp
PSGL-Ig -- American Home Products	Renal cancer vaccine -- Macropharm
PSP-94 -- Procyon	repifermin -- Human Genome Sciences
PTH 1-34 -- Nobex	Respiratory syncytial virus PFP-2 vaccine -- Wyeth-Lederle
Quilimmune-M -- Antigenics	Respiratory syncytial virus vaccine -- GlaxoSmithKline, Pharmacia, Pierre Fabre
R 744 -- Roche	Respiratory syncytial virus vaccine inactivated
R 101933	Respiratory syncytial virus-parainfluenza virus vaccine -- Aventis Pasteur, Pharmacia
R 125224 -- Sankyo	Reteplase -- Boehringer Mannheim, Hoffman La-Roche
RA therapy -- Cardion	Retropep -- Retroscreen
Rabies vaccine recombinant -- Aventis Pasteur, BioChem Vaccines, Kaketsuken Pharmaceuticals	RFB4 (dsFv) PE38
RadioTheraCIM -- YM BioSciences	RFI 641 -- American Home Products
Ramot project No. 1315 -- Ramot	RFTS -- UAB Research Foundation
Ramot project No. K-734A -- Ramot	RG 12986 -- Aventis Pasteur
Ramot project No. K-734B -- Ramot	RG 83852 -- Aventis Pasteur
Ranibizumab (Anti-VEGF fragment) -- Genentech	RG-1059 -- RepliGen
RANK -- Immunex	rGCR -- NIH
ranpirnase -- Alfacell	rGLP-1 -- Restoragen
ranpirnase-anti-CD22 MAb -- Alfacell	rGRF -- Restoragen
RANTES inhibitor -- Milan	rh Insulin -- Eli Lilly
RAPID drug delivery systems -- ARIAD	RHAMM targeting peptides -- Cangene
rasburicase -- Sanofi	rHb1.1 -- Baxter Intl.
rBPI-21, topical -- XOMA	rhCC10 -- Claragen
RC 529 -- Corixa	rhCG -- Serono
rCFTR -- Genzyme Transgenics	Rheumatoid arthritis gene therapy

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Rheumatoid arthritis vaccine -- Veterans Affairs Medical Center	SB RA 31012 --
rhLH -- Serono	SC 56929 -- Pharmacia
Ribozyme gene therapy -- Genset	SCA binding proteins -- Curis, Enzon
Rickettsial vaccine recombinant	scFv(14E1)-ETA Berlex Laboratories,
RIGScan CR -- Neoprobe	Schering AG
RIP-3 -- Rigel	ScFv(FRP5)-ETA --
Rituximab -- Genentech	ScFv6C6-PE40 --
RK-0202 -- RxKinetix	SCH 55700 -- Celltech
RLT peptide -- Esperion	Schistosomiasis vaccine -- Glaxo
rM/NEI -- IVAX	Wellcome/Medeva, Brazil
rmCRP -- Immtech	SCPF -- Advanced Tissue Sciences
RN-1001 -- Renovo	scuPA-suPAR complex -- Hadasit
RN-3 -- Renovo	SD-9427 -- Pharmacia
RNAse conjugate -- Immunomedics	SDF-1 -- Ono
RO 631908 -- Roche	SDZ 215918 -- Novartis
Rotavirus vaccine -- Merck	SDZ 280125 -- Novartis
RP 431 -- DuPont Pharmaceuticals	SDZ 89104 -- Novartis
RP-128 -- Resolution	SDZ ABL 364 -- Novartis
RPE65 gene therapy --	SDZ MMA 383 -- Novartis
RPR 110173 -- Aventis Pasteur	Secretin -- Ferring, Repligen
RPR 115135 -- Aventis Pasteur	serine protease inhibs -- Pharis
RPR 116258A -- Aventis Pasteur	sermorelin acetate -- Serono
rPSGL-Ig -- American Home Products	SERP-1 -- Viron
r-SPC surfactant -- Byk Gulden	sertenef -- Dainippon
RSV antibody -- Medimmune	serum albumin, Recombinant human --
Ruplizumab -- Biogen	Aventis Behring
rV-HER-2/neu -- Therion Biologics	serum-derived factor -- Hadasit
SA 1042 -- Sankyo	Sevirumab -- Novartis
sacrosidase -- Orphan Medical	SGN 14 -- Seattle Genetics
Sant 7	SGN 15 -- Seattle Genetics
Sargramostim -- Immunex	SGN 17/19 -- Seattle Genetics
saruplase -- Gruenthal	SGN 30 -- Seattle Genetics
Satumomab -- CytoGen	SGN-10 -- Seattle Genetics
SB 1 -- COR Therapeutics	SGN-11 -- Seattle Genetics
SB 207448 -- GlaxoSmithKline	SH 306 -- DuPont Pharmaceuticals
SB 208651 -- GlaxoSmithKline	Shanvac-B -- Shantha
SB 240683 -- GlaxoSmithKline	Shigella flexneri vaccine -- Avant, Acambis, Novavax
SB 249415 -- GlaxoSmithKline	Shigella sonnei vaccine --
SB 249417 -- GlaxoSmithKline	sICAM-1 -- Boehringer Ingelheim
SB 6 -- COR Therapeutics	Salteplase -- Genzyme

FIG. 28Z

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SIV vaccine -- Endocon, Institut Pasteur	Staphylococcus aureus vaccine conjugate -- Nabi
SK 896 -- Sanwa Kagaku Kenkyusho	Staphylococcus therapy -- Tripep
SK-827 -- Sanwa Kagaku Kenkyusho	Staphylokinase -- Biovation, Prothera, Thrombogenetics
Skeletex -- CellFactors	Streptococcal A vaccine -- M6
SKF 106160 -- GlaxoSmithKline	Pharmaceuticals, North American Vaccine
S-nitroso-AR545C --	Streptococcal B vaccine -- Microscience
SNTP -- Active Biotech	Streptococcal B vaccine recombinant -- Biochem Vaccines
somatomedin-1 -- GroPep, Mitsubishi-Tokyo, NIH	Streptococcus pyogenes vaccine
somatomedin-1 carrier protein -- Insmed	STRL-33 -- NIH
somatostatin -- Ferring	Subalin -- SRC VB VECTOR
Somatotropin/	SUIS -- United Biomedical
Human Growth Hormone -- Bio-Tech, General, Eli Lilly	SUIS-LHRH -- United Biomedical
somatropin -- Bio-Tech, General, Alkermes, ProLease, Aventis Behring, Biovector, Cangene, Dong-A, Eli Lilly, Emisphere, Enact, Genentech, Genzyme Transgenics, Grandis/InfiMed, CSL, InfiMed, MacroMed, Novartis, Novo Nordisk, Pharmacia Serono, TranXenoGen	SUN-E3001 -- Suntory
somatropin derivative -- Schering AG	super high affinity monoclonal antibodies -- YM BioSciences
somatropin, AIR -- Eli Lilly	Superoxide dismutase -- Chiron, Enzon, Ube Industries, Bio-Tech, Yeda
Somatropin, inhaled -- Eli Lilly/Alkermes	superoxide dismutase-2 -- OXIS
somatropin, Kabi -- Pharmacia	supressin -- UAB Research Foundation
somatropin, Orasome -- Novo Nordisk	SY-161-P5 -- ThromboGenics
Sonermin -- Dainippon Pharmaceutical	SY-162 -- ThromboGenics
SP(V5.2)C -- Supertek	Systemic lupus erythematosus vaccine -- MedClone/VivoRx
SPf66	T cell receptor peptides -- Xoma
sphingomyelinase -- Genzyme	T cell receptor peptide vaccine
SR 29001 -- Sanofi	T4N5 liposomes -- AGI Dermatics
SR 41476 -- Sanofi	TACI, soluble -- ZymoGenetics
SR-29001 -- Sanofi	targeted apoptosis -- Antisoma
SS1(dsFV)-PE38 -- NeoPharm	tasonermin -- Boehringer Ingelheim
$\beta$ 2 microglobulin -- Avidex	TASP
$\beta$ 2-microglobulin fusion proteins -- NIH	TASP-V
$\beta$ -amyloid peptides -- CeNeS	Tat peptide analogues -- NIH
$\beta$ -defensin -- Pharis	TBP I -- Yeda
Staphylococcus aureus infections -- Inhibitex/ZLB	TBP II
	TBV25H -- NIH
	Tc 99m ior cea1 -- Center of Molecular Immunology
	Tc 99m P 748 -- Diatide

FIG. 28AA

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Tc 99m votumumab -- Intracell	Tissue factor -- Genentech
Tc-99m rh-Annexin V -- Theseus Imaging	Tissue factor pathway inhibitor
teceleukin -- Biogen	TJN-135 -- Tsumura
tenecteplase -- Genentech	TM 27 -- Avant
Teriparatide -- Armour Pharmaceuticals, Asahi Kasei, Eli Lilly	TM 29 -- Avant
terlipressin -- Ferring	TMC-151 -- Tanabe Seiyaku
testisin -- AMRAD	TNF tumour necrosis factor -- Asahi Kasei
Tetrafibrin -- Roche	TNF Alpha -- CytImmune
TFPI -- EntreMed	TNF antibody -- Johnson & Johnson
tgD-IL-2 -- Takeda	TNF binding protein -- Amgen
TGF-Alpha -- ZymoGenetics	TNF degradation product -- Oncotech
TGF- $\beta$ -- Kolon	TNF receptor -- Immunex
TGF- $\beta$ 2 -- Insmed	TNF receptor 1, soluble -- Amgen
TGF- $\beta$ 3 -- OSI	TNF Tumour necrosis factor-alpha -- Asahi Kasei, Genetech, Mochida
Thalassaemia gene therapy -- Crucell	TNF-Alpha inhibitor -- Tripep
TheraCIM-h-R3 -- Center of Molecular Immunology, YM BioSciences	TNFR:Fc gene therapy -- Targeted Genetics
Theradigm-HBV -- Epimmune	TNF-SAM2
Theradigm-HPV -- Epimmune	ToleriMab -- Innogenetics
Theradigm-malaria -- Epimmune	Toxoplasma gondii vaccine -- GlaxoSmithKline
Theradigm-melanoma -- Epimmune	TP 9201 -- Telios
TheraFab -- Antisoma	TP10 -- Avant
ThGRF 1-29 -- Theratechnologies	TP20 -- Avant
ThGRF 1-44 -- Theratechnologies	tPA -- Centocor
Thrombin receptor activating peptide -- Abbott	trafermin -- Scios
thrombomodulin -- Iowa, Novocastra	TRAIL/Apo2L -- Immunex
Thrombopoietin -- Dragon Pharmaceuticals, Genentech	TRAIL-R1 MAb -- Cambridge Antibody Technologies
thrombopoietin, Pliva -- Receptron	transferrin-binding proteins -- CAMR
Thrombospondin 2 --	Transforming growth factor-beta-1 -- Genentech
thrombostatin -- Thromgen	transport protein -- Genesis
thymalfasin -- SciClone	Trastuzumab -- Genetech
thymocartin -- Gedeon Richter	TRH -- Ferring
thymosin Alpha1 -- NIH	Triabin -- Schering AG
thyroid stimulating hormone -- Genzyme	Triconal
tICAM-1 -- Bayer	Triflavin
Tick anticoagulant peptide -- Merck	troponin I -- Boston Life Sciences
TIF -- Xoma	TRP-2 <sup>A</sup> -- NIH
Tifacogin -- Chiron, NIS, Pharmacia	trypsin inhibitor -- Mochida

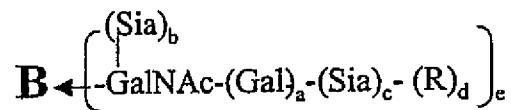
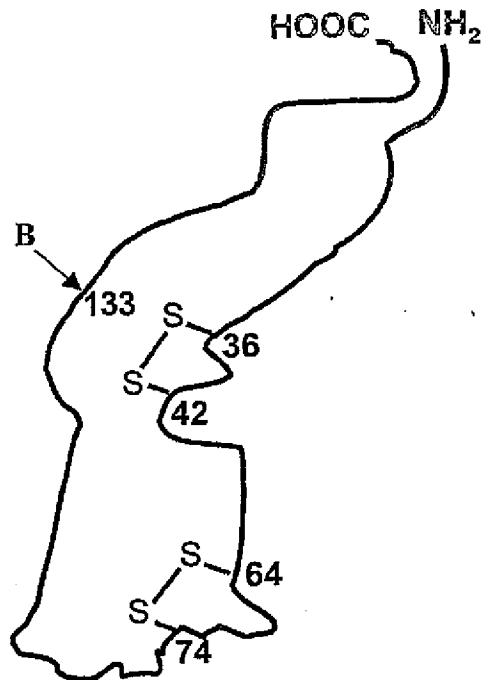
FIG. 28BB

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TSP-1 gene therapy –	Vascular endothelial growth factors – R&D
TT-232	Systems
TTS-CD2 – Active Biotech	vascular targeting agents -- Peregrine
Tuberculosis vaccine -- Aventis Pasteur, Genesis	vasopermeation enhancement agents -- Peregrine
Tumor Targeted Superantigens – Active Biotech -- Pharmacia	vasostatin – NIH
tumour vaccines – PhotoCure	VCL – Bio-Tech. General
tumour-activated prodrug antibody conjugates -- Millennium/ImmunoGen	VEGF – Genentech, Scios
tumstatin – ILEX	VEGF inhibitor – Chugai
Tuvirumab – Novartis	VEGF-2 -- Human Genome Sciences
TV-4710 – Teva	VEGF-Trap -- Regeneron
TWEAK receptor -- Immunex	viscumin, recombinant -- Madaus
TXU-PAP	Vitaxin
TY-10721 – TOA Eiyo	Vitrase -- ISTA Pharmaceuticals
Type I diabetes vaccine -- Research Corp	West Nile virus vaccine -- Bavarian Nordic
Typhoid vaccine CVD 908	WP 652
U 143677 -- Pharmacia	WT1 vaccine -- Corixa
U 81749 -- Pharmacia	WX-293 – Wilex BioTech.
UA 1248 – Arizona	WX-360 -- Wilex BioTech.
UGIF – Sheffield	WX-UK1 – Wilex BioTech.
UIC 2	XMP-500 – XOMA
UK 101	XomaZyme-791 -- XOMA
UK-279276 – Corvas Intl.	XTL 001 – XTL Biopharmaceuticals
urodilatin – Pharis	XTL 002 -- XTL Biopharmaceuticals
urofollitrophin – Serono	yeast delivery system -- Globalimmune
Urokinase -- Abbott	Yersinia pestis vaccine
uteroferrin-- Pepgen	YIGSR-Stealth -- Johnson & Johnson
V 20 -- GLYCODesign	Yissum Project No. D-0460 -- Yissum
V2 vasopressin receptor gene therapy vaccines -- Active Biotech	YM 207 -- Yamanouchi
Varicella zoster glycoprotein vaccine -- Research Corporation Technologies	YM 337 -- Protein Design Labs
Varicella zoster virus vaccine live -- Cantab Pharmaceuticals	Yttrium-90 labelled biotin
Vascular endothelial growth factor -- Genentech, University of California	Yttrium-90-labeled anti-CEA MAb T84.66 --
	ZD 0490 – AstraZeneca
	ziconotide -- Elan
	ZK 157138 -- Berlex Laboratories
	Zolimomab aritox
	Zorcell – Immune Response
	ZRXL peptides -- Novartis

FIG. 28CC

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a-c, e (independently selected) = 0 or 1;  
d = 0;  
R = modifying group, sialyl or  
oligosialyl

FIG. 29A

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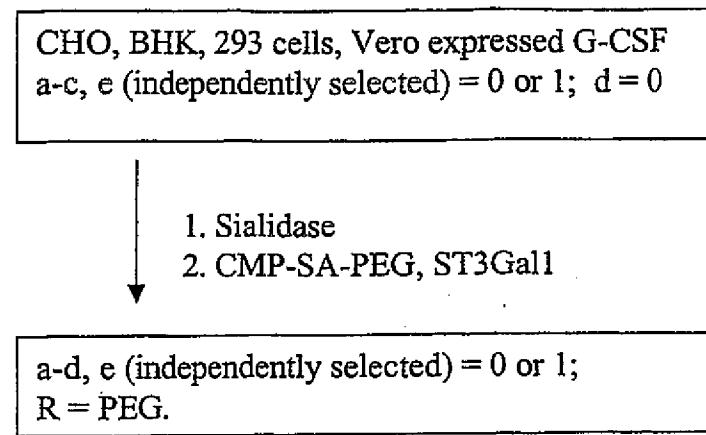


FIG. 29B

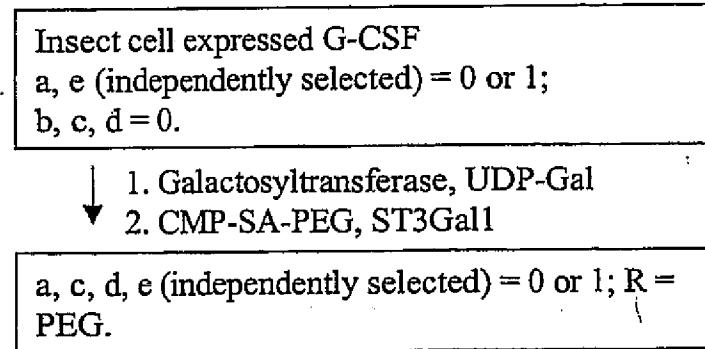


FIG. 29C

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E. coli expressed G-CSF  
a-e = 0.

↓  
1. GalNAc Transferase, UDP-GalNAc  
2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1;  
a, b = 0; R = PEG.

FIG. 29D

NSO expressed G-CSF  
a, e (independently selected) = 0 or 1;  
b, c, d = 0

↓  
1. CMP-SA-levulinate, ST3Gal1  
2. H<sub>4</sub>N<sub>2</sub>-PEG

a, c, d, e (independently selected) = 0 or 1;  
b = 0; R = PEG.

FIG. 29E

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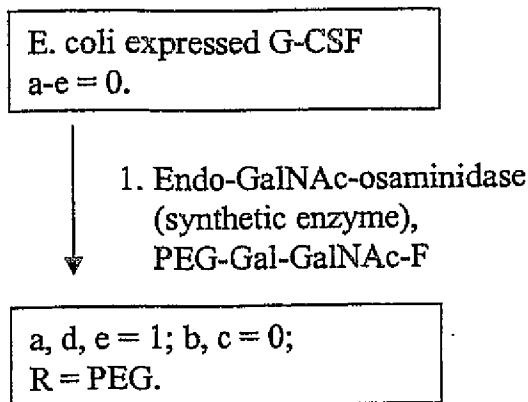


FIG. 29F

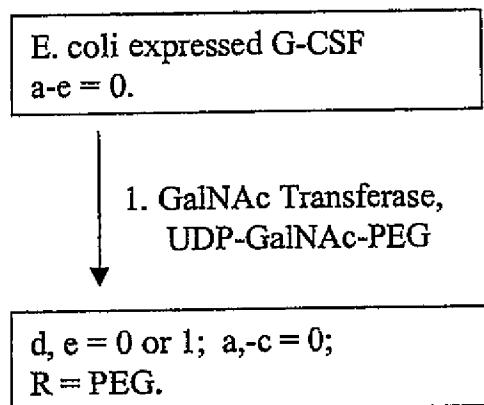
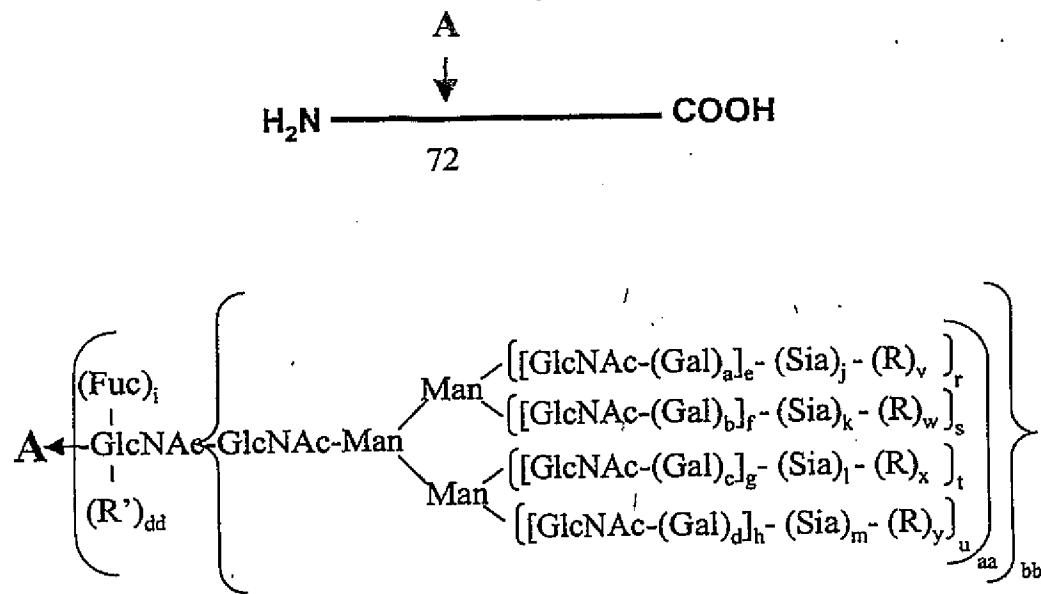


FIG. 29G

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a-d, i, n-u (independently selected) = 0 or 1.  
 aa, bb, cc, dd, ee (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 6.  
 j-m (independently selected) = 0 to 20.  
 v-z = 0; R = modifying group, mannose, oligo-mannose.  
 R' = H, glycosyl residue, modifying group,  
 glycoconjugate.

FIG. 30A

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CHO, BHK, 293 cells, Vero expressed  
interferon alpha 14C.  
a-d, aa, bb = 1; e-h = 1 to 4;  
cc, j-m, i, r-u (independently selected) = 0 or 1;  
q, n-p, v-z, cc, dd, ee = 0.

1. Sialidase  
2. CMP-SA-PEG, ST3Gal3

a-d, aa, bb = 1; e-h = 1 to 4;  
bb, cc, i, r-u (independently selected) = 0 or 1;  
q, n-p, v-z, cc, dd, ee = 0;  
v-y (independently selected) = 1,  
when j-m (independently selected) = 1;  
R = PEG.

FIG. 30B

Insect cell or fungi expressed interferon alpha-14C.  
a-d, f, h, j-q, s, u, v-z, cc, dd, ee = 0;  
e, g, i, r, t (independently selected) = 0 or 1;  
aa, bb = 1.

1. GNT's 1&2, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal-PEG

b, d, f, h, j-q, s, u, w, y, z, cc, dd, ee = 0;  
a, c, e, g, i, r, t, v, x (independently selected) = 0 or 1;  
v, x (independently selected) = 1,  
when a, c, (independently selected) = 1;  
aa, bb = 1; R = PEG.

FIG. 30C

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Yeast expressed interferon alpha-14C.  
a-q, cc, dd, ee, v-z = 0;  
r-y (independently selected) = 0 to 1;  
aa, bb = 1;  
R (branched or linear) = Man, oligomannose or  
polysaccharide.

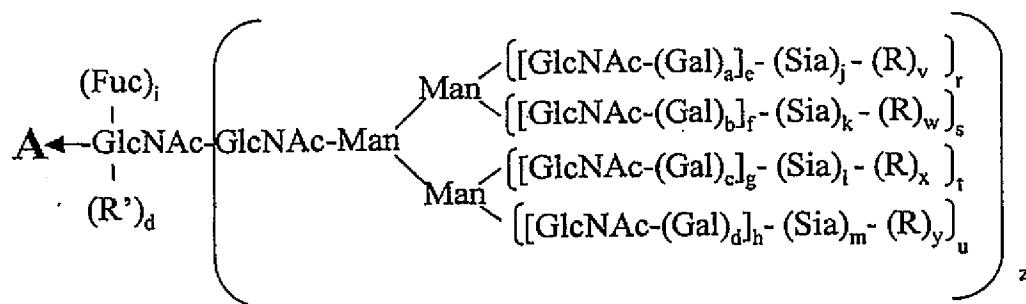
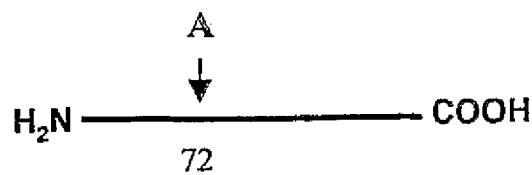
↓

1. Endo-H
2. Galactosyltransferase, UDP-Gal
- 3.. CMP-SA-PEG, ST3Gal3

a-z, bb = 0; aa = 1; R' = -Gal-Sia-PEG.

FIG. 30D

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a-d, i, r-u (independently selected) = 0 or 1.  
 e-h (independently selected) = 0 to 4.  
 j-m (independently selected) = 0 or 1.  
 n, v-y = 0; z = 0 or 1.  
 R = polymer; R' = sugar, glycoconjugate.

FIG. 30E

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CHO, BHK, 293 cells, Vero expressed  
interferon alpha-14C.  
h = 1 to 3;  
a-g, j-m, i (independently selected) = 0 or 1;  
r-u (independently selected) = 0 or 1;  
n, v-y = 0; z = 1.

↓ 1. CMP-SA-PEG, ST3Gal3

h = 1 to 3;  
a-g, i (independently selected) = 0 or 1;  
r-u (independently selected) = 0 or 1;  
j-m, v-y (independently selected) = 0 or 1;  
z = 1; n = 0; R = PEG.

FIG. 30F

Insect cell or fungi expressed  
interferon alpha-14C.  
a-d, f, h, j-n, s, u, v-y = 0;  
e, g, i, r, t (independently selected) = 0 or 1;  
z = 1.

↓ 1. GNT's 1,2,4,5, UDP-GlcNAc  
2. Galactosyltransferase, UDP-Gal  
3. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1;  
z = 1; n = 0; R = PEG.

FIG. 30G

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Yeast expressed interferon alpha-14C.  
a-n = 0; r-y (independently selected) = 0 to 1;  
z = 1; R (branched or linear) = Man,  
oligomannose.

1. mannosidases  
2. GNT's 1,2,4,5, UDP-GlcNAc  
3. Galactosyltransferase, UDP-Gal  
4.. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1;  
z = 1; n = 0; R = PEG.

FIG. 30H

NSO expressed interferon alpha 14C.  
a-i, r-u (independently selected) = 0 or 1;  
j-m, n, v-y = 0; z = 1.

1. CMP-SA-levulinate, ST3Gal3,  
buffer, salt  
2. H<sub>4</sub>N<sub>2</sub>-PEG

a-i, j-m, r-y (independently selected) = 0 or 1;  
n = 0; z = 1; R = PEG.

FIG. 30I